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**MACROCYCLIC IMMUNOMODULATORS WITH NOVEL
CYCLOHEXYL RING REPLACEMENTS**

5 This is continuation-in-part of U.S. patent application Serial No. 159,406, filed November 30, 1993.

Technical Field

10 The present invention relates to novel chemical compounds having immunomodulatory activity, and in particular to macrolide immunosuppressants. More particularly, the invention relates to semisynthetic analogs of ascomycin and FK-506, intermediates and processes for their preparation, pharmaceutical compositions containing such compounds and methods of treatment employing the same.

15 **Background of the Invention**

 The compound cyclosporine (cyclosporin A) has found wide use since its introduction in the fields of organ transplantation and immunomodulation, and has brought about a significant increase in the success rate for transplantation procedures. Unsatisfactory side-effects associated with cyclosporine, however, such as nephrotoxicity, 20 have led to a continued search for immunosuppressant compounds having improved efficacy and safety.

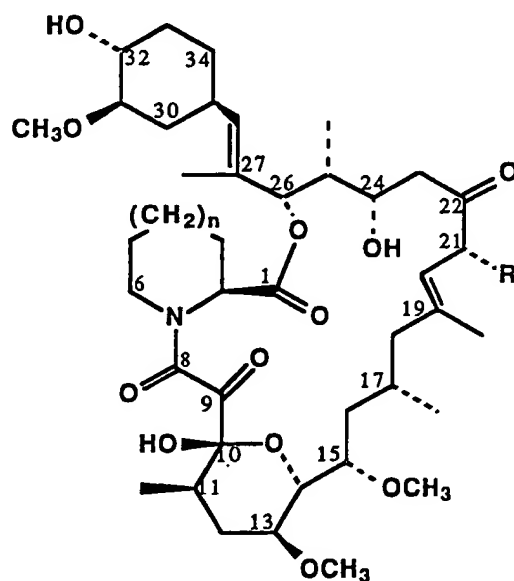
 Recently, several classes of macrocyclic compounds having potent immunomodulatory activity have been discovered. Okuhara *et al.*, in European Patent Application No. 184,162, published June 11, 1986, disclose a number of macrocyclic 25 compounds isolated from the genus *Streptomyces*. Immunosuppressant FK-506, isolated from a strain of *S. tsukubaensis*, is a 23-membered macrocyclic lactone represented by formula 1a, below. Other related natural products, such as FR-900520 (1b) and FR-900523 (1c), which differ from FK-506 in their alkyl substituent at C-21, have been isolated from *S. hygroscopicus yakushimnaensis*. Yet another analog, FR-900525, 30 produced by *S. tsukubaensis*, differs from FK-506 in the replacement of a pipercolic acid moiety with a proline group.

 FR-900520, also known as ascomycin, has been previously disclosed by Arai *et al.* in U.S. Patent No. 3,244,592, issued April 5, 1966, where the compound is described as an antifungal agent. Monaghan, R.L., *et al.*, on the other hand, describe the use of

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ascomycin as an immunosuppressant in European Patent Application No. 323,865, published July 12, 1989.

Although the immunosuppressive activity of FK-506 has been clinically confirmed, toxicity in mammals has limited its utility. The activity of FK-506 has, however, prompted efforts to discover novel analogs of FK-type compounds which possess superior properties. These efforts include the isolation of new fermentation products, the microbial transformation of existing chemical entities, the chemical modification of these macrocycles, and the synthesis of hybrid species derived from smaller synthetic fragments.



(1)

- | | |
|-----------------|--|
| I(a): FK-506 | R = CH ₂ CH=CH ₂ ; n=1 |
| I(b): FR-900520 | R = CH ₂ CH ₃ ; n=1 |
| I(c): FR-900523 | R = CH ₃ ; n=1 |
| I(d): FR-900525 | R = CH ₂ CH=CH ₂ ; n=0 |

Fermentation products of FK-type compounds include C-21-epi derivatives of FK-506; a 31-demethylated derivative of FK-506; 31-oxo-FK-506; and compounds derived from FK-506, FR-900523 and FR-900525 which are characterized by the introduction of hydroxy-protecting groups, formation of a double bond by elimination of

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water between carbons 23 and 24, oxidation of the hydroxy group at carbon 24 to the ketone, and reduction of the allyl side-chain at carbon 21 via hydrogenation. Other published derivatives include those derived from FK-506 and FR-900520 where the lactone ring is contracted to give a macrocyclic ring containing two fewer carbons.

5 Several microbial transformations of FK-type compounds at carbon 13 have been published, such as the microbial demethylation of FR-900520 to form the *bis*-demethylated 13,31-dihydroxy ring-rearranged derivative of FR-900520; the microbial monodemethylation of FK-506 and FR-900520, respectively; and the microbial demethylation of FR-900520 at C-31, as well as a number of other macrocyclic microbial
10 transformation products.

Numerous chemical modifications of the FK-type compounds have been attempted. These include the preparation of small synthetic fragments of FK-type derivatives; a thermal rearrangement of a variety of derivatives of FK-506 which expands the macrocyclic ring by two carbons; and modifications which include methyl ether
15 formation at C-32 and/or C-24, oxidation of the C-32 alcohol to the ketone, and epoxide formation at C-9.

Although some of these modified compounds exhibit immunosuppressive activity, the need remains for macrocyclic immunosuppressants which do not have the serious side effects frequently associated with immunosuppressant therapy. Accordingly, one object of
20 the invention is to provide novel semisynthetic macrolides which possess the desired immunomodulatory activity but which may be found to minimize untoward side effects.

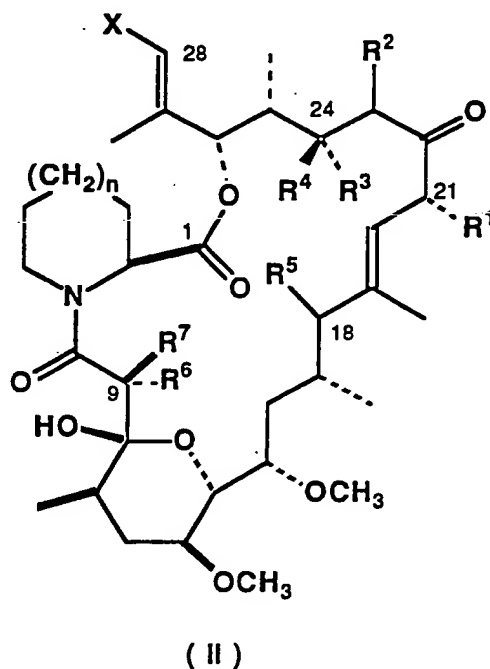
Another object of the present invention is to provide synthetic processes for the preparation of such compounds from starting materials obtained by fermentation, as well as chemical intermediates useful in such synthetic processes.

25 A further object of the invention is to provide pharmaceutical compositions containing, as an active ingredient, at least one of the above compounds. Yet another object of the invention is to provide a method of treating a variety of immune system-related disease states, including post-transplant tissue rejection and autoimmune
30 disfunction.

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Disclosure of the Invention

In one aspect of the present invention are disclosed compounds having the formula:



5

wherein n is zero or one;

10 R¹ is selected from the group consisting of methyl, ethyl, propyl, cyclopropylmethyl, 2-oxopropyl, 2-ethanal, 2-hydroxyethyl and allyl;

R² is hydrogen or R² taken together with R³ forms a C-22/C-23 bond;

15 R³ is selected from the group consisting of hydrogen, hydroxy and protected hydroxy; or R² taken together with R³ forms a C-22/C-23 bond; or R³ taken together with R⁴ is oxo;

R⁴ is hydrogen or R⁴ taken together with R³ is oxo;

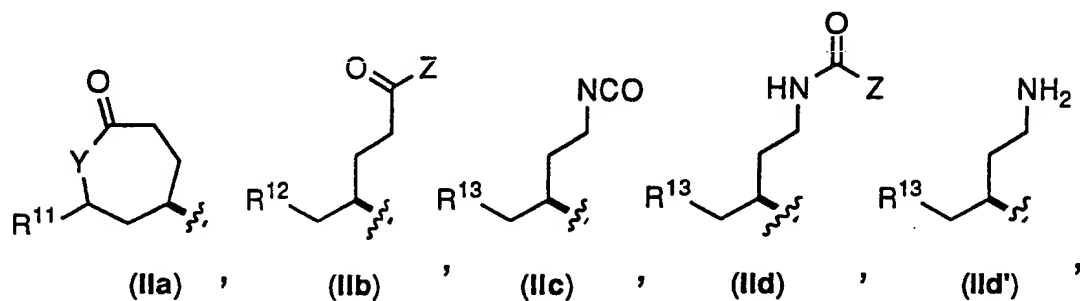
R⁵ is selected from hydrogen, hydroxy, protected hydroxy and fluoro;

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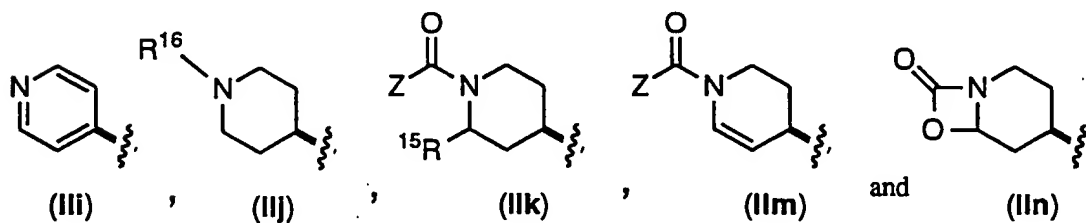
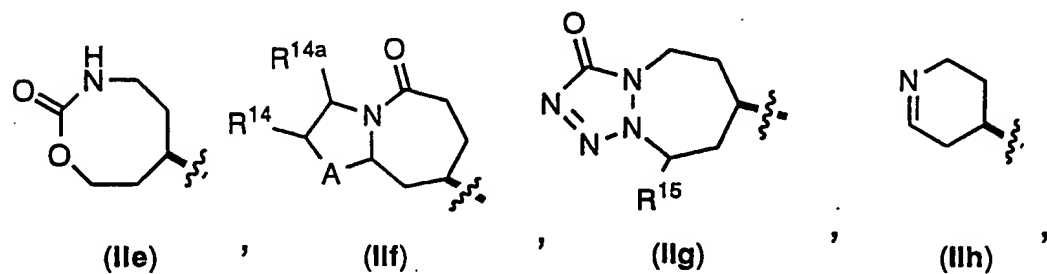
R^6 and R^7 are independently selected from hydrogen, hydroxy and protected hydroxy, with the proviso that at least one of R^6 and R^7 is hydrogen; or R^6 and R^7 taken together are oxo;

5

X is a substituent selected from the group of radicals having the following subformulae:



10



15

wherein A is -O- or -S-;

Y is -O- or -NR²¹, wherein R²¹ is selected from the group consisting of:

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- (1) hydrogen,
(2) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,
(3) aryl and
5 (4) heterocyclic;

Z is selected from the group consisting of hydrogen, hydroxy, -(C1-C10-alkyl), aryl, heterocyclic, -(C1-C10-alkoxy) and -NR²³R²⁴, wherein R²³ and R²⁴ are independently selected from:

- 1 0 (1) hydrogen,
(2) aryl,
(3) heterocyclic and
(4) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,

1 5

or, alternatively, -NR²³R²⁴ represents a 3- to 7-membered heterocyclic ring, with the proviso that in subformulae (II_d), (II_k) and (II_m) Z is other than hydroxy;

R¹¹ is hydrogen, hydroxy or methoxy;

2 0

R¹² is selected from the group consisting of:

- (1) -CHO,
(2) -(C1-C10-alkyl) optionally substituted with one, two or three substituents independently selected from the group consisting of hydroxy, amino, aryl and heterocyclic,
2 5 (3) -(C2-C10-alkenyl) optionally substituted with aryl or heterocyclic,
(4) -CH₂-NR²¹R²² where R²¹ is independently defined as above and R²² is hydrogen or acyl;

- 3 0 R¹³ is selected from the group consisting of -CHO, hydroxy, protected hydroxy, -(C2-C10-alkenyl) optionally substituted with aryl and -(C1-C10-alkyl) optionally substituted with aryl, with the proviso that in subformula (II_d) R¹³ is other than -CHO;

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R¹⁴ and R^{14a} are independently selected from hydrogen, aryl, heterocyclic and -(C1-C10-alkyl) or, taken together with the carbon atoms to which they are attached, R¹⁴ and R^{14a} form an aryl group or a heterocyclic group;

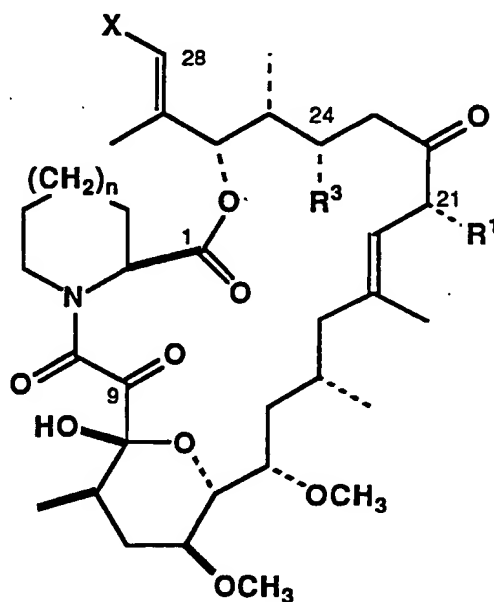
5 R¹⁵ is hydrogen or hydroxy; and

R¹⁶ is selected from the group consisting of

- (1) hydrogen,
- (2) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or
- 10 heterocyclic,
- (3) aryl,
- (4) heterocyclic and
- (5) -C(=NH)-NH₂;

or a pharmaceutically acceptable salt, ester, amide or prodrug thereof.

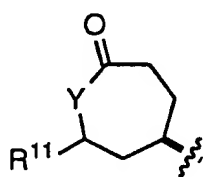
15 Preferred among the compounds of the present invention are those represented by formula (III):



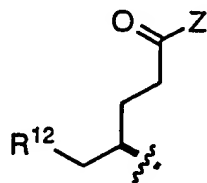
(III)

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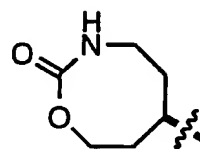
wherein n is one, R^1 and R^3 are defined as above and X is a substituent selected from the group of radicals having the following subformulae:



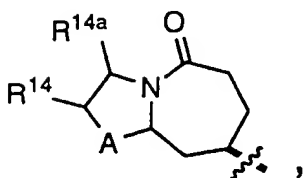
(IIa)



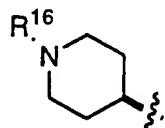
(IIb)



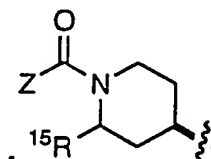
(Ile)



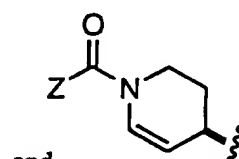
(IIf)



(IIj)



(IIk)



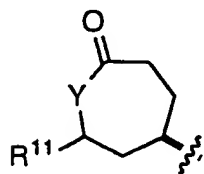
(IIlm)

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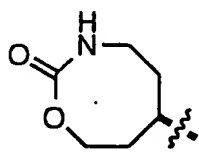
wherein A , Y , Z , R^{12} , R^{14} , R^{14a} and R^{16} are defined as above and R^{11} and R^{15} are each hydrogen.

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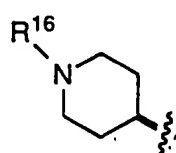
Particularly preferred among the compounds of the present invention are those represented by formula III in which n is one and X is a substituent selected from the group of radicals having the following subformulae:



(IIa)

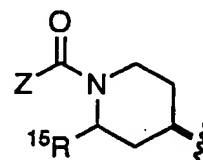


(Ile)



(IIj)

and



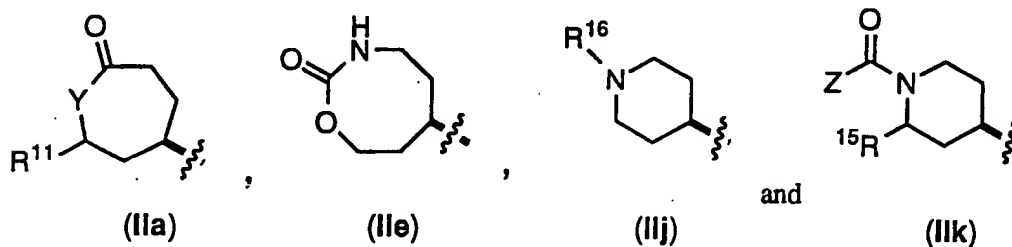
(IIk)

15

wherein Y , Z and R^{16} are defined as above and R^{11} and R^{15} are each hydrogen.

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More particularly preferred among the compounds of the present invention are those represented by formula III in which n is one and X is a substituent selected from the group of radicals having the following subformulae:



wherein Y is -O-,

Z is -(C1-C10-alkyl), aryl, heterocyclic or -NR²³R²⁴, wherein R²³ and R²⁴ are independently selected from:

- (1) hydrogen,
- (2) aryl,
- (3) heterocyclic and
- (4) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,

or, alternatively, -NR²³R²⁴ represents a 3- to 7-membered heterocyclic ring, R¹⁶ is

- (1) hydrogen,
- (2) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,
- (3) aryl or
- (4) heterocyclic,

and R¹¹ and R¹⁵ are each hydrogen.

Especially preferred, and regarded as the best mode of carrying out the present invention, are the compounds prepared in Examples 11, 37 and 56 (described below).

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When examined for immunomodulatory activity using a common *in vitro* biological assay, the compounds of the invention are seen to be potent immunosuppressant agents. The compounds of this invention possess immunosuppressive, antimicrobial, antifungal, antiviral, antiinflammatory, and antiproliferative activity, as well as the ability to reverse
5 chemotherapeutic drug resistance. Moreover, the compounds of the invention possess the ability to reverse chemotherapeutic drug resistance. As agents which block T-cell activation, a prerequisite for HIV proliferation, the compounds are useful as prophylactics for the prevention of HIV replication. While, the compounds of the invention are useful when used independently of other agents, combination therapy with other
10 immunosuppressants is beneficial as well. These other immunosuppressant agents include but are not limited to FK-506, rapamycin, cyclosporin A, mycophenolic acid, azathioprine, prednisolone, cyclophosphamide, brequinar and leflunomide.

Accordingly, in another aspect of the present invention are disclosed pharmaceutical compositions comprising a compound of the invention in combination with
15 a pharmaceutically acceptable carrier. Suitable carriers and methods of formulation are also disclosed.

In a further aspect of the present invention are disclosed processes for the preparation of the above compounds and synthetic intermediates useful in the preparations of these and other immunomodulator derivatives of ascomycin.

20 In yet another aspect of the present invention is disclosed a method of immunomodulatory treatment in a human or lower mammal, comprising the administration of a therapeutically effective amount of at least one compound of the invention to a patient in need of such treatment.

25 Throughout this specification and in the appended claims, the following terms have the meanings specified:

The term "acyl" as used herein refers to $-C(O)R^{30}$ wherein R^{30} is aryl, (C1-C10-alkyl), (C2-C10-alkenyl) or heterocyclic. Examples include, but are not limited to, acetyl, pivaloyl, benzoyl and the like.

30 The term "C2-C10-alkenyl" as used herein refers to a straight or branched chain group of 2 to 10 carbon atoms containing at least one carbon-carbon double bond including, but not limited to, ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl and the like.

The term "C1-C6-alkoxy" as used herein refers to $-OR^{31}$ wherein R^{31} is

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(C1-C6-alkyl) including, but not limited to, methoxy, ethoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy and the like.

The term "C1-C10-alkyl" as used herein refers to a monovalent straight chain or branched chain group of 1 to 10 carbon atoms including, but not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl and the like.

The term "C2-C10-alkynyl" as used herein refers to a straight or branched chain group of 2 to 10 carbon atoms containing at least one carbon-carbon triple bond including, but not limited to, propargyl, 1-propynyl, 1-butenyl, 2-butenyl and the like.

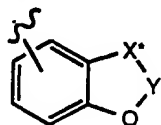
The term "aryl", as used herein, refers to mono-, bi- or tricyclic carbocyclic ring system having one or two aromatic rings. Examples of aryl include, but are not limited to, phenyl, 1- or 2-naphthyl, fluorenyl, (1,2)-dihydronaphthyl, (1,2,3,4)-tetrahydronaphthyl, indenyl and the like. Aryl groups can be unsubstituted or substituted with one, two or three substituents independently selected from the group consisting of -(C1-to-C10-alkyl), -(C2-to-C10-alkenyl), halogen, $-(CH_2)_mN(C1-C6-alkyl)_2$, wherein m is zero to six, -CN, -CHO, mono-, di-, tri-, or perhalogenated -(C1-C6-alkyl), $-S(O)_p(C1-C6-alkyl)$, wherein p is 0, 1 or 2, $-C(O)N(C1-C6-alkyl)$, $-(CH_2)_mO(C1-C6-alkyl)$, where m is as defined above, $-(CH_2)_qOC(O)(C1-C6-alkyl)$, wherein q is zero to six, $-(CH_2)_rC(O)O(C1-C6-alkyl)$ wherein r is zero to six, $-NO_2$, $-N_3$, $-S(O)_2N(C1-C6-alkyl)_2$, $-(C2-to-C10-alkynyl)$, $-C\equiv C-Si(CH_3)_3$, guanidino, hydroxy, -COOH, -(C1-C6-alkoxy), $-OC(O)(C1-C6-alkyl)$, unsubstituted aryl and unsubstituted Het.

The term "cyclo(C3-C10-alkyl)" as used herein refers to a cyclic group of 3 to 10 carbons including, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "heterocyclic ring" or "heterocyclic" or "heterocycle" as used herein refers to any 3- or 4-membered ring containing a heteroatom selected from oxygen, nitrogen and sulfur; or a 5-, 6- or 7-membered ring containing one, two or three nitrogen atoms; one oxygen atom; one sulfur atom; one nitrogen and one sulfur atom; one nitrogen and one oxygen atom; two oxygen atoms in non-adjacent positions; one oxygen and one sulfur atom in non-adjacent positions; or two sulfur atoms in non-adjacent positions. The 5-membered ring has 0-2 double bonds and the 6- and 7-membered rings have 0-3 double bonds. The nitrogen heteroatoms can be optionally quaternized. The term "heterocyclic" also includes bicyclic groups in which any of the above heterocyclic rings is fused to a benzene ring or a cyclohexane ring or another heterocyclic ring (for example, indolyl,

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quinolyl, isoquinolyl, tetrahydroquinolyl, benzofuryl, dihydrobenzofuryl or benzothienyl and the like). Heterocyclics include: azetidiny, oxetanyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidiny, homopiperidiny, pyrazinyl, piperazinyl, pyrimidinyl, 5 pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiomorpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, indolyl, quinoliny, isoquinoliny, benzimidazolyl, benzothiazolyl, benzoxazolyl, furyl, thienyl, thiazolidinyl, isothiazolyl, triazolyl, tetrazolyl, isoxazolyl, oxadiazolyl, thiadiazolyl, pyrrolyl, pyrimidyl, cytosinyl, thiosytosinyl, xanthenyl, xanthonyl, uricyl, thyminy and benzothienyl.



10 Heterocyclics also include compounds of the formula where X* is -CH₂- or -O- and Y* is -C(O)- or [-C(R'')₂-]_v where R'' is hydrogen or C₁-C₄-alkyl and v is 1, 2 or 3 such as 1,3-benzodioxolyl, 1,4-benzodioxanyl and the like.

Heterocyclics can be unsubstituted or monosubstituted or disubstituted with substituents independently selected from -(C₁-to-C₁₀-alkyl), -(C₂-to-C₁₀-alkenyl), 15 halogen, amino, -(CH₂)_fN(C₁-C₆-alkyl)₂ wherein f is zero to six, -CN, -CHO, mono-, di-, tri-, or perhalogenated -(C₁-C₆-alkyl), -S(O)_g(C₁-C₆-alkyl) wherein g is 0, 1 or 2, -C(O)N(C₁-C₆-alkyl), -(CH₂)_hO(C₁-C₆-alkyl) wherein h is 0 to 6, -(CH₂)_iOC(O)(C₁-C₆-alkyl) wherein i is 0 to 6, -(CH₂)_jC(O)O(C₁-C₆-alkyl) wherein j is 0 to 6, -NO₂, -N₃, -S(O)₂N(C₁-C₆-alkyl)₂, -(C₂-to-C₁₀-alkynyl), -C[Si(CH₃)₃], 20 guanidino, hydroxy, -COOH, -(C₁-C₆-alkoxy), -OC(O)(C₁-C₆-alkyl), oxo (=O), unsubstituted aryl and unsubstituted Het.

The term "protected hydroxy group" as used herein refers to a hydroxy group which has been protected by a group which is known in the art of organic synthesis to protect a hydroxyl group against undesirable reaction during synthetic procedures and to be selectively removable such as those hydroxy protecting groups disclosed in T.W. 25 Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2nd ed., John Wiley & Son, Inc., 1991, which is hereby incorporated herein by reference. Examples of hydroxy protecting groups include, but are not limited to, substituted methyl ethers, for example, methoxymethyl, methylthiomethyl, 2-methoxyethoxymethyl, benzyloxymethyl, 30 2-(trimethylsilyl)ethoxymethyl, t-butyl, benzyl and triphenylmethyl; tetrahydropyranyl ethers; substituted ethyl ethers, for example, 2,2,2-trichloroethyl; silyl ethers, for example,

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dimethylhexylsilyl, trisubstituted silyl such as tris(loweralkyl)silyl (*e.g.*, trimethylsilyl, triethylsilyl, tributylsilyl, tri-isopropylsilyl, *tert*-butyldimethylsilyl, tri-*tert*-butylsilyl, triphenylsilyl, triphenylmethyldimethylsilyl, etc.), loweralkyldiarylsilyl (*e.g.*, methyldiphenylsilyl, ethyldiphenylsilyl, propyldiphenylsilyl, *tert*-butyldiphenylsilyl, etc.), triarylsilyl (*e.g.*, triphenylsilyl, trixylylsilyl, etc.) and triarylalkylsilyl (*e.g.*, tribenzylsilyl, etc.); -C(O)H; -C(O)-loweralkyl (for example, acetyl, propionyl, pivaloyl, *t*-butylacetyl and the like); -C(O)-aryl (for example, benzoyl and the like); alkoxycarbonyl (for example, ethoxycarbonyl and the like); -S(O)₂-(loweralkyl); -S(O)₂-(aryl); and the like.

The term "oxo" as used herein refers to (=O).

1 0 The term "pharmaceutically acceptable salts, esters, amides and prodrugs" as used herein refers to those carboxylate salts, amino acid addition salts, esters, amides and prodrugs of the compounds of the present invention which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, or the like, commensurate
1 5 with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention, which may be prepared *in situ* during the final isolation and purification of the compounds or by separately reacting the purified compound in its free
2 0 base form with a suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate and laurylsulphonate salts and the
2 5 like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like (see, for example S. M. Berge, et al., "Pharmaceutical Salts," J. Pharm. Sci., 66:1-19 (1977)).
3 0

Examples of pharmaceutically-acceptable, non-toxic esters of the compounds of this invention include C1-to-C6-alkyl esters wherein the alkyl group is a straight or branched chain. Acceptable esters also include C5-to-C7-cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl. C1-to-C4 alkyl esters are preferred.

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Esters of the compounds of the present invention may be prepared according to conventional methods. Conversely, non-toxic esters of alcoholic moieties on the compounds of the invention may be constructed by condensing these alcohols with C1-to-C6-alkyl carboxylic acids, C1-to-C6-alkyl dicarboxylic acids or aryl-carboxylic acids.

5 Examples of such esters include, but are not limited to acetyl, benzoyl or hemi-succinyl.

Examples of pharmaceutically-acceptable, non-toxic amides of the compounds of this invention include amides derived from ammonia, primary C1-to-C6-alkyl amines and secondary di-C1-to-C6-alkyl amines. In the case of secondary amines the amine may also be in the form of a 5-or- 6 membered heterocycle containing one nitrogen atom. Amides
1 0 derived from ammonia, C1-to-C3-alkyl primary amides and di-C1-to-C2-alkyl secondary amides are preferred. Amides of the compounds of the invention may be prepared according to conventional methods.

"Prodrug" refers to compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formula, for example, by hydrolysis in blood. A thorough
1 5 discussion is provided in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems", Vol 14 of the A.C.S. Symposium Series, and in *Bioreversible Carriers in Drug Design*, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, which is hereby incorporated herein by reference.

Prodrugs of compounds of the present invention may be prepared by any suitable
2 0 method. For those compounds in which the prodrug moiety is an amino acid or peptide functionality, the condensation of the drug's amino group with amino acids and peptides may be effected in accordance with conventional condensation methods such as the azide method, the mixed acid anhydride method, the DCC (dicyclohexylcarbodiimide) method, the active ester method (p-nitrophenyl ester method, N-hydroxysuccinic acid imide ester
2 5 method, cyanomethyl ester method and the like), the Woodward reagent K method, the DCC-HOBT (1-hydroxy-benzotriazole) method and the like. Classical methods for amino acid condensation reactions are described in "Peptide Synthesis" Second Edition, M. Bodansky, Y.S. Klausner and M.A. Ondetti (1976).

As in conventional peptide synthesis, branched chain amino and carboxyl groups at
3 0 alpha and omega positions in amino acids may be protected and deprotected if necessary. The protecting groups for amino groups which can be used involve, for example, benzyloxycarbonyl (Z or Cbz), o-chlorobenzyloxycarbonyl ((2-Cl)Z)), p-nitrobenzyloxycarbonyl (Z(NO₂)), p-methoxybenzyloxycarbonyl (Z(OMe)), t-amylloxycarbonyl (Aoc), isobornealoxycarbonyl, adamantyloxycarbonyl (Adoc),

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2-(4-biphenyl)-2-propyloxy carbonyl (Bpoc), 9-fluorenyl-methoxycarbonyl (Fmoc), methylsulfonylethoxy carbonyl (Msc), trifluoroacetyl, phthalyl, formyl, 2-nitrophenylsulfonyl (Nps), diphenylphosphinothioyl (Ppt), dimethylphosphino-thioyl (Mpt) and the like.

5 The examples for protecting groups for carboxyl groups involve, for example, benzyl ester (OBzl), cyclohexyl ester, 4-nitrobenzyl ester (OBzlNO₂), t-butyl ester (OtBu), 4-pyridylmethyl ester (OPic) and the like.

10 In the course of the synthesis of certain of the compounds of the present invention, specific amino acids having functional groups other than amino and carboxyl groups in the branched chain such as arginine, cysteine, serine and the like may be protected, if necessary, with suitable protecting groups. It is preferable that, for example, the guanidino group (NG) in arginine may be protected with nitro, p-toluenesulfonyl (Tos), benzyloxycarbonyl (Z), adamantyloxycarbonyl (Adoc), p-methoxybenzene-sulfonyl, 4-methoxy-2,6-dimethyl-benzenesulfonyl (Mts) and the like; the thiol group in cysteine 15 may be protected with benzyl, p-methoxybenzyl, triphenylmethyl, acetamidomethyl, ethylcarbamy, 4-methylbenzyl (4-MeBzl), 2,4,6-trimethylbenzyl (Tmb) and the like; and the hydroxy group in serine may be protected with benzyl (Bzl), t-butyl, acetyl, tetrahydropyranyl (THP) and the like.

20 As disclosed above, the compounds of the invention are those described by the general formula (II), as well as the pharmaceutically acceptable salts, esters amides and prodrugs thereof. The compounds of the invention are formed by modification of FR-900520 (ascomycin) or one of its congeners (such as FK-506, etc.) by oxidative cleavage at the C-32 position with optional modifications exercised at the C-9, C-18, C-23 and/or 25 C-24 positions. Multiple modifications are also possible by the careful selection of syntheses from those disclosed herein, as by the use of other synthetic methods known to those skilled in the art.

30 The compounds of the invention may be prepared using one or more processes. The starting materials for use in these processes are preferably one of the macrolides isolated from culture media obtained in accordance with known methods by fermentation of microorganisms of the genus Streptomyces, which are disclosed in European Patent Application No. 0184162. Samples are available from the Fermentation Research Institute, Tsukuba, Ibaraki 305, Japan under the provisions of the Budapest Treaty, under

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- deposit No. FERM BP-927. This strain has been redeposited on April 27, 1989 with the Agricultural Research Culture Collection International Depository, Peoria, Illinois 61604, USA under the provisions of the Budapest Treaty, under deposit No. NRRL 18488. The macrolide FR-900520 (European Patent Application 0184162), also known as ascomycin,
- 5 may be prepared in accordance to the published methods of (i) H. Hatanaka, M. Iwami, T. Kino, T. Goto and M. Okuhara, *FR-900520 and FR-900523, Novel immunosuppressants isolated from A streptomyces. I. Taxonomy of the producing strain. J. Antibiot.*, 1988, XLI(11), 1586-1591; (ii) H. Hatanaka, T. Kino, S. Miyata, N. Inamura, A. Kuroda, T. Goto, H. Tanaka and M. Okuhara, *FR-900520 and FR-900523, Novel*
- 10 *immunosuppressants isolated from A streptomyces. II. Fermentation, isolation and physico-chemical and biological characteristics. J. Antibiot.*, 1988, XLI(11), 1592-1601; (iii) T. Arai, Y. Koyama, T. Suenaga and H. Honda, *Ascomycin, An Antifungal Antibiotic. J. Antibiot.*, 1962. 15(231-2); and (iv) T. Arai in U.S. Patent No. 3,244,592. The macrolide FR-900506 (U.S. Patent No. 4,929,611. issued May 29, 1990), also
- 15 known as FK-506, may be prepared in accordance to the published methods of Tanaka, Kuroda, and co-workers, *J. Am. Chem. Soc.*, 1987, 109, 5031. One or more of the processes discussed below may be then employed to produce the desired compound of the invention.
- 20 Such processes comprise:
- (a) producing a compound of formula IIa, where Y is O and R¹¹ is -OMe by selective oxidation of a selected -C(=O)-CH(OMe)- group in a corresponding compound;
- (b) producing a compound of formula IIa, where Y is O and R¹¹ is hydrogen, by recyclization of a compound of formula IIb, where R¹² is -CH₂-OH and Z is OH;
- 25 (c) producing a compound of formula IIa, where Y is O and R¹¹ is OH (which also exists in the form of formula IIb, where R¹² is aldehyde and Z is OH) by acid catalyzed ring opening of a compound of formula IIa, where Y is O and R¹¹ is -OMe;
- (d) producing a compound of formula IIb where R¹² is R²¹-NH-CH₂- by reductive alkylation of a R²¹-NH₂ and a compound of formula IIb where R¹² is -CHO;
- 30 (e) producing a compound of formula IIa, where R¹¹ is hydrogen and Y is N-R²¹ by cyclic amide formation of a compound of formula IIb, where R¹² is R²¹-NH-CH₂-;
- (f) producing a compound of formula IIb, where R¹² is -CH₂-OH, by selective reduction of an aldehyde group;

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- (g) producing a compound of formula IIb, where Z is $-NR^{23}R^{24}$ by amide formation of a carboxyl group with an appropriate amine by amide formation reactions;
- (h) producing a compound of formula IIc by activation of a $-COOH$ group with azides and Curtius rearrangement of a compound of formula IIb;
- 5 (i) producing a compound of formula IId where Z is R^{21} by reaction of an isocyanate of a selected compound IIc, with $R^{21}-CO_2H$;
- (j) producing a compound of formula IId where Z is $-(C1-C10-alkoxy)$ by reaction of an isocyanate of a selected compound IIc with a corresponding alcohol;
- (k) producing a compound of formula IId, where Z is $NR^{23}R^{24}$ by reaction of an
10 isocyanate of a selected compound IIc with a corresponding amine;
- (l) producing a compound of formula IIe by reductive cyclization of an appropriate aldehyde and isocyanate of a selected compound;
- (m) producing a compound of formula IIe by reaction of an amino alcohol or thioamino alcohol with a compound of formula IIb, where R^{12} is an aldehyde and Z is
15 $-OH$, followed by intramolecular amide formation reaction;
- (n) producing a compound of formula IIg by reaction of sodium azide with a compound of formula IIc where R^{13} is an aldehyde;
- (o) producing a compound of formula IIh by reaction of a compound of formula IIc, where R^{13} is an aldehyde, with hydrofluoric acid;
- 20 (p) producing a compound of formula Iii by oxidation of a compound of formula IIh;
- (q) producing a compound of formula IIj by reduction of a corresponding cyclic imine IIh;
- (r) producing a compound of formula IIrn by dehydration reaction of a selected
25 compound of formula IId; and
- (s) producing a compound of formula IIIk by selective hydrogenation of a compound of formula IIrn.

30 In process (a), a suitable reagent for Baeyer-Villiger oxidation is an organic or inorganic peracid such as m-chloroperbenzoic acid, trifluoroperacetic acid, nitro-perbenzoic acid, monopermaleic acid, monoperphthalic acid, performic acid or peracetic acid etc. (Krow, G.R. *Organic Reactions*, Vol. 43, pp251-798, John Wiley & Sons, Inc., 1993). The oxidation may be carried in a solvent which does not adversely affect the reaction (e.g. methylene chloride, chloroform, carbon tetrachloride, dichloroethane or chlorobenzene).

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The reaction may require cooling or heating (°C to 100 °C), depending on the method used. Further, the reaction is preferably conducted in the presence of an inorganic base such as cesium carbonate, cesium bicarbonate, potassium carbonate, potassium bicarbonate and the like. The reaction may require 20 minutes to 24 hours to complete, depending on the reagents chosen.

5 In process (b), a suitable reagent for lactonization is Mukaiyama reagent (Mukaiyama T., *Challenges in Synthetic Organic Chemistry*, p.118, Clarendon Press, Oxford, 1990), alkyl chloroformate, carbodiimide, diphenylphosphoryl azide or carbonyldiimidazole. The intramolecular cyclization may be carried out in a solvent which
1 0 does not adversely affect the reaction (e.g. tetrahydrofuran, methylene chloride, chloroform, N,N-dimethylformamide, N-methylpyrrolidone, pyridine or a mixture thereof). The reaction may require cooling or heating, depending on the method used. Further, the reaction is preferably conducted in the presence of an organic or inorganic base such as cesium carbonate, lutidine, picoline, quinoline, diisopropylethylamine and the
1 5 like. The reaction temperature is preferably from -30 °C to 60 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent used.

In process (c), a suitable acid catalyst is p-toluenesulfonic acid, methanesulfonic acid, trifluoroacetic acid, fluorosulfonic acid, hydrofluoric acid, hydrochloric acid, formic acid, acetic acid and the like. The reaction may be carried out in acetone, acetaldehyde,
2 0 diethylketone, n-butaldehyde and the like. The reaction may require cooling or heating, depending on the method used.

In process (d), a suitable reducing agent is trialkylsilane such as triethylsilane, hydrogen in the presence of catalyst, sodium cyanoborohydride and the like. The reaction may be carried in a solvent which does not adversely affect the reaction (e.g.
2 5 tetrahydrofuran, N,N-dimethylformamide, N-methylpyrrolidone, formic acid, acetic acid or a mixture thereof). The reaction may require cooling or heating, depend on the reagents used. Further, the reaction is preferably conducted in the presence of amine, R²¹-NH₂, in the presence of an organic acid such as formic acid, acetic acid, trifluoroacetic acid and the like.

3 0 In process (e), a suitable reagent for lactamization is Mukaiyama reagent (Mukaiyama T., *Challenges in Synthetic Organic Chemistry*, p.118, Clarendon Press, Oxford, 1990), alkyl chloroformate, carbodiimide, diphenylphosphoryl azide or carbonyldiimidazole. The intramolecular cyclization may be carried out in a solvent which does not adversely affect the reaction (e.g. tetrahydrofuran, methylene chloride,

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chloroform, N,N-dimethylformamide, N-methylpyrrolidone, pyridine or a mixture thereof). The reaction may require cooling or heating, depending on the method used. Further, the reaction is preferably conducted in the presence of an organic or inorganic base such as cesium carbonate, lutidine, picoline, quinoline, diisopropylethylamine and the like. The reaction temperature is preferably from -30 °C to 60 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent used.

In process (f), a suitable reducing reagent is sodium borohydride, sodium cyanoborohydride, lithium triacetoxyborohydride, L-selectride and the like. The reaction may be carried out in a solvent which does not adversely affect the reaction (e.g. diethyl ether, tetrahydrofuran, N,N-dimethylformamide or a mixture thereof). The reaction temperature is preferably from -78 °C to 40 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent used.

In process (g), the condensation of the amino group with carboxylic acid may be affected in accordance with conventional condensation methods such as the azide method, the mixed acid anhydride method, the carbodiimide method, the active ester method (p-nitrophenyl ester method, N-hydroxysuccinic acid imide ester method, cyanomethyl ester method and the like), the Woodward reagent K method, the DCC-HOBt (dichlorohexylcarbodiimide-1-hydroxybenzotriazole), Mukaiyama method and the like. Classical methods for amide bond formation reactions are described in "Peptide Synthesis: Second Edition, M.Bodansky, Y.S.Klausner and M.A. Ondetti, 1976).

In process (h), the carboxylic acid group is first activated with chloroformate and converted to acyl azide with sodium azide. Alternatively, the carboxylic acid is directly converted to acyl azide using diphenylphosphoryl azide. The acyl azide may be used in Curtius rearrangement with or without further purification. The preferred solvent for the acyl azide formation is acetone-water, tetrahydrofuran-water, dioxane-water, tetrahydrofuran, dichloromethane, chloroform, chlorobenzene and the like. The Curtius rearrangement reaction may be carried in a solvent which does not adversely affect the reaction (e.g. toluene, tetrahydrofuran, dioxane, chloroform or a mixture thereof). The reaction temperature is preferably from 25 °C to 100 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent chosen.

In process (i), the carboxylic acid, R²¹-COOH, is directly reacted with the isocyanate in a solvent which does not adversely affect the reaction (e.g. toluene, chlorobenzene, chloroform, methylene chloride or a mixture thereof). The reaction

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temperature is preferably from 25 °C to 100 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent chosen.

5 In process (j), the alcohol, (C1-C10-alkyl) alcohol, is directly reacted with the isocyanate in a solvent which does not adversely affect the reaction (e.g. toluene, chlorobenzene, chloroform, methylene chloride or a mixture thereof). The reaction temperature is preferably from 25 °C to 100 °C. Further, the reaction is carried out in the presence of organic base such as diisopropylethylamine, triethylamine, pyridine, lutidine and the like. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent chosen.

1 0 In process (k), the amine, $R^{23}R^{24}NH$ is directly reacted with the isocyanate in a solvent which does not adversely affect the reaction (e.g. toluene, chlorobenzene, chloroform, methylene chloride or a mixture thereof). The reaction temperature is preferably from 25 °C to 100 °C. Further, the reaction is carried out in the presence of organic base such as diisopropylethylamine, triethylamine, pyridine, lutidine and the like.
1 5 The reaction may require 20 minutes to 24 hours to complete, depending on the reagent chosen.

In process (l), a suitable reducing reagent is sodium borohydride, sodium cyanoborohydride, lithium triacetoxyborohydride, L-selectride and the like. The reaction may be carried out in a solvent which does not adversely affect the reaction (e.g. diethyl ether, tetrahydrofuran, N,N-dimethylformamide or a mixture thereof). The reaction temperature is preferably from -78 °C to 40 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent used.
2 0

In process (m), an amino alcohol or thioamino alcohol is reacted directly with a compound of subformula IIb (R^{12} is -CHO, Z is -OH) in the presence of a drying agent.
2 5 The reaction may be carried in a solvent which does not adversely affect the reaction (e.g. tetrahydrofuran, chloroform, dichloroethane, methylene chloride, chlorobenzene, toluene, benzene or a mixture thereof). Suitable solid drying agents are powdered anhydrous magnesium sulfate, molecular sieves and the like. The intermediate is then cyclized by a standard amide bond formation reaction. The condensation of the amino group with
3 0 carboxylic acid may be affected in accordance with conventional condensation methods such as the azide method, the mixed acid anhydride method, the carbodiimide method, the active ester method (p-nitrophenyl ester method, N-hydroxysuccinic acid imide ester method, cyanomethyl ester method and the like), the Woodward reagent K method, the DCC-HOBt (dichlorohexylcarbodiimide-1-hydroxybenzotriazole), Mukaiyama method and

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the like. Classical methods for amide bond formation reactions are described in "Peptide Synthesis: Second Edition, M. Bodansky, Y.S. Klausner and M.A. Ondetti, 1976).

5 In process (n), the carboxylic acid group is first activated with chloroformate and converted to acyl azide with excess sodium azide. Alternatively, the carboxylic acid is directly converted to acyl azide using excess diphenylphosphoryl azide. The acyl azide may be used in Curtius rearrangement followed by cyclization reaction without further purification. The preferred solvent for the acyl azide formation is acetone-water, tetrahydrofuran-water, dioxane-water, tetrahydrofuran, dichloromethane, chloroform, chlorobenzene and the like. The Curtius rearrangement reaction may be carried in a solvent which does not adversely affect the reaction (e.g. toluene, tetrahydrofuran, dioxane, chloroform or a mixture thereof). The reaction temperature is preferably from 25 °C to 100 °C. The reaction may require 20 minutes to 24 hours to complete, depending on the reagent chosen.

1 5 In process (o), a compound of subformulae IIc (R^{13} is -CHO) is reacted with one to five equivalents of 48% hydrofluoric acid in a solvent which does not adversely affect the reaction (e.g. tetrahydrofuran, acetonitrile, acetone, dioxane or a mixture thereof). The reaction temperature is preferably -78 to 0 °C. The reaction may require 1 to 24 hours to complete. At the end of the reaction, the hydrofluoric acid is neutralized by an inorganic base such as cesium carbonate, cesium bicarbonate, potassium bicarbonate and the like.

2 0 In process (p), a suitable catalyst for aromatization is palladium on carbon or platinum. A suitable oxidizing agent is air, oxygen, an olefin and the like. The reaction is preferably carried out in a solvent which does not adversely affect the reaction (e.g. tetrahydrofuran, acetone, diethyl ether, dioxane or a mixture thereof). The reaction may take 1 to 14 days to complete, depending on the conditions chosen.

2 5 In process (q), a suitable reducing agent is trialkylsilane such as triethylsilane, hydrogen in the presence of catalyst, sodium cyanoborohydride and the like. The reaction may be carried in a solvent which does not adversely affect the reaction (e.g. tetrahydrofuran, N,N-dimethylformamide, N-methylpyrrolidone, formic acid, acetic acid or a mixture thereof). The reaction may require cooling or heating, depend on the reagents used. Further, the reaction is preferably conducted in the presence of amine, R^{21} -NH₂, in the presence of an organic acid such as formic acid, acetic acid, trifluoroacetic acid and the like.

3 0 In process (r), a compound of subformula IId (R^{13} is -CHO), is activated with methanesulfonyl chloride, trifluoromethanesulfonyl anhydride, fluorosulfonyl anhydride

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and the like in the presence of an organic base. The preferred organic base is tri-alkyl amine such as triethylamine, diisopropylethylamine, pyridine, lutidine and the like. The dehydration reaction is preferably carried in a solvent which does not adversely affect the reaction (e.g. methylene chloride, chloroform, dichloroethane, chlorobenzene or a mixture thereof). The reaction may require cooling or heating, depending on the conditions used.

In process (s), a suitable reducing agent is trialkylsilane such as triethylsilane, hydrogen in the presence of catalyst, sodium cyanoborohydride and the like. The reaction may be carried in a solvent which does not adversely affect the reaction (e.g. tetrahydrofuran, N,N-dimethylformamide, N-methylpyrrolidone, formic acid, acetic acid or a mixture thereof). The reaction may require cooling or heating, depend on the reagents used. Further, the reaction is preferably conducted in the presence of amine, $R^{21}-NH_2$, in the presence of an organic acid such as formic acid, acetic acid, trifluoroacetic acid and the like.

It should be noted that numerous asymmetric centers may exist in the compounds of the present invention. Except where otherwise specified, the present invention contemplates the various stereoisomers and mixtures thereof. It should also be noted that certain variable elements of the structural formulae herein, such as the radical R^{21} or the subscript integers m and s, may appear more than once in a particular formula. In such instances, it is intended that, within a single formula, the values of these variables may be the same or different at each occurrence.

The present invention can be illustrated by the following non-limiting, representative examples.

Example 1.

Formula IIa: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together $=O$; $Y=O$; $R^{11}=OCH_3$.

Example 1a. Formula I(b): $n=1$; $R=-CH_2CH_3$; 24, 32-Bis-tert-butyldimethylsiloxy-ascornycin.

A solution of imidazole (41.4 g) in N,N-dimethylformamide (200 mL) was added dropwise into a stirred solution of ascornycin (60 g) and tert-butyldimethylsilyl chloride (91.7 g) in N,N-dimethylformamide (200 mL) at 0 °C. After being stirred at 0 °C for 2 hours, the reaction mixture was stirred at room temperature for 48 hours. N,N-

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dimethylformamide and excess tert-butyldimethylsilyl chloride was removed by distillation under high vacuum. The bath temperature was kept below 35 °C during the distillation. The residue was partitioned between ether (250 mL) and water (400 mL). The aqueous phase was extracted with ether (3x150 mL). The combined organic phase was washed with 1 N hydrochloric acid (3x150 mL), brine (300 mL), dried over magnesium sulfate and solvent removed *in vacuo*. The product was purified by silica gel chromatography (1.25 kg) eluting with 12% acetone in hexanes. Yield: 70.3 g; MS (FAB) *m/z*: M+H = 1022.

10 Example 1b. Formula I(b): n=1; R = -CH₂CH₃; 24-tert-Butyldimethylsiloxy-ascomycin. A solution of hydrofluoric acid (0.5 mL of 48% hydrofluoric acid in 10 mL of acetonitrile) was added dropwise into a solution of the title compound of Example 1(a) (16 g) in acetonitrile (50 mL) at 0 °C. After being stirred at this temperature for an additional 15 minutes, the cooling bath was removed and the reaction mixture was stirred at room
15 temperature for 1 hour. The reaction mixture was cooled to 0 °C and powdered sodium bicarbonate (5 g) was added followed by powdered magnesium sulfate (10 g). After being stirred at 0 °C for 1 hour, solids were filtered off and solvent removed *in vacuo*. The residue was purified by silica gel chromatography (15 g) eluting ether. The crude product was further purified by silica gel chromatography (100 g) eluting with 10% acetone in
20 hexanes. Yield: 12.3 g; MS (FAB) *m/z*: M+K = 944.

Example 1c. Formula I(b): n=1; R = -CH₂CH₃; 24-tert-Butyldimethylsiloxy-32-desoxy-32-oxo-ascomycin.
Dimethylsulfoxide (3.26 mL) was added into a stirred solution of oxalyl chloride (3.5 g) in
25 dry methylene chloride (20 mL) at -78 °C under nitrogen. After being stirred at -78 °C for 0.5 hour, a solution of the title compound of Example 1 (b) (8.3 g) in dry methylene chloride (30 mL) was added dropwise and stirred at this temperature for an additional 0.5 hour. Triethylamine (9 mL) in methylene chloride (30 mL) was added dropwise into the reaction mixture at -78 °C. The cooling bath was removed after the addition and the
30 reaction mixture stirred at room temperature for 0.5 hour. Anhydrous ether (200 mL) was added into the reaction mixture and precipitations filtered off. The solids was triturated with more ether (200 mL). The combined filtrate was washed once with 1 N hydrochloric acid, brine, dried over magnesium sulfate and solvent removed *in vacuo*. The product was

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purified by silica gel chromatography (50 g) eluting with 10% acetone/hexanes followed by 20% acetone/hexanes. Yield: 7.7 g; MS (FAB) m/z : $M+K = 942$.

Example 1d. Formula I(b): $n=1$; $R = -CH_2CH_3$; 32-desoxy-32-oxo-ascomycin.

- 5 The title compound was prepared from the title compound of Example 1(c) (6 g) and hydrofluoric acid in acetonitrile according to the procedure described in Example 1(b). Yield: 5.0 g; MS (FAB) m/z : $M+H = 790$.

- 10 Example 1e. Formula IIa: $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OH$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; $Y = O$; $R^{11} = OCH_3$.

- Cesium carbonate (powdered, 15.5 g) was added into a stirred solution of the title compound of Example 1(d) (9.5 g) in dichloromethane (100 mL) at 0 °C. *m*-Chloroperbenzoic acid (5.6 g, 50%) was added portionwise into the stirred suspension over 10 minutes. After being stirred at 0 °C for two hours, the solids were removed by
15 filtration. The filtrate was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic phase was washed once with brine, dried over magnesium sulfate and solvent removed *in vacuo*. The crude residue was purified by silica gel (125 g) chromatography eluting with 30% acetone in hexanes. Yield: 6.4 g; MS (FAB) m/z : $M+K = 844$.

20

Example 2.

Formula IIa: $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OTBS$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; $Y = O$; $R^{11} = OCH_3$, where TBS is tert-butyldimethylsilyl

- 25 The title compound was prepared from *m*-chloroperbenzoic acid and the title compound of Example 1(c) in the presence of cesium carbonate according to the procedure described in Example 1. MS (FAB) m/z : $M+K = 958$.

Example 3.

- 30 Formula IIb: $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OH$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; $Z = OH$; $R^{12} = -CHO$.

p-Toluenesulfonic acid mono hydrate (0.03 g) was added into a stirred solution of the title compound of Example 1 (6.4 g) in acetone (130 mL) and stirred at room temperature for 0.5 hour. Solvent was removed *in vacuo*. The solid residue was redissolved in acetone (130 mL) and stirred at room temperature for 2 hours. The process was repeated and

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stirring continued for an additional 3 hours. Solvent was removed *in vacuo*. The solid was purified by silica gel chromatography eluting with 70% acetone in hexanes. Yield: 6.4 g; MS (FAB) m/z : $M+K = 830$.

5 Example 4.

Formula IIb: $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OTBS$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; $Z = OH$; $R^{12} = -CHO$.

The title compound was prepared from the title compound of Example 2 and *p*-toluenesulfonic acid in acetone according to the procedure described in Example 3. MS
10 (FAB) m/z : $M+K = 944$.

Example 5.

Formula IIb: $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OTBS$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; $Z = OH$; $R^{12} = -CH_2OH$.

15 L-selectride (13.6 mL, 1M in THF) was added dropwise over 30 minutes into a stirred solution of the title compound of Example 4 (7.6 g) in 100 mL of tetrahydrofuran (THF) at -78 °C. After being stirred at -78 °C for 1 hour, 1N HCl (aq) was added into the reaction mixture to destroy excess reducing agent. After the addition of hydrochloric acid, the reaction mixture was allowed to stir at 0 °C for 15 minutes. The reaction mixture was
20 partitioned between ether and 1 N HCl (aq). The organic phase was washed once with brine, dried over magnesium sulfate and solvent removed *in vacuo*. The solid residue was purified by silica gel chromatography (90 g) eluting with 5% isopropanol in dichloromethane. Yield: 4.65 g; MS (FAB) m/z : $M+K = 946$.

25 Example 6.

Formula IIb: $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OTBS$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; $Z = NHBn$; $R^{12} = -CH_2OH$.

Bn: benzyl

A solution of the title compound of Example 5 (0.6 g), 1-hydroxybenzotriazole (0.09 g),
30 benzylamine (0.15 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.19 g) in *N,N*-dimethylformamide (5 mL) was stirred at room temperature overnight. The reaction mixture was partitioned between 1:1 ether/ethyl acetate solution and water. The organic phase was washed once with brine, dried over magnesium sulfate

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and solvent removed *in vacuo*. The product was purified by silica gel chromatography (5 g) eluting with 40% acetone in hexanes. Yield: 0.56 g.

Example 7.

5 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=NHBn$; $R^{12}=-CH_2OH$.

A solution of HF (0.09 mL of 48% aq HF dissolved in 4.5 mL of acetonitrile) was added into a stirred solution of the title compound of Example 6 (0.45 g) in acetonitrile (0.5 mL) at 0 °C. After being stirred at room temperature for 4
1 0 hours, the reaction was cooled to 0 °C and powdered sodium bicarbonate (1 g) was added and stirred for an additional hour. Solid was removed by filtration and solvent removed *in vacuo*. The solid was purified by silica gel chromatography (12 g) eluting with 35% acetone in hexanes. Yield: 0.24 g; MS (FAB) m/z : $M+K = 921$. m.p. 150-159°C.
1 5 ^{13}C NMR: (CDCl₃-2 rotamers)
213.2, 212.6, 196.0, 192.6, 172.8, 169.4, 169.2, 165.7, 164.4, 139.6, 138.9, 138.5, 138.5, 133.6, 133.3, 131.8, 130.3, 128.6, 128.0, 127.9, 127.3, 123.5, 123.3, 98.7, 97.6, 80.5, 79.5, 77.4, 77.2, 77.0, 76.6, 75.4, 73.7, 72.9, 72.5, 69.3, 68.3, 65.8, 61.0, 60.9, 57.7, 57.2, 56.4, 56.2, 56.0, 55.2, 54.9, 53.0,
2 0 49.1, 48.5, 45.6, 44.0, 43.7, 43.6, 43.6, 40.2, 39.6, 39.4, 38.7, 38.7, 35.3, 34.5, 34.0, 33.9, 33.8, 33.7, 32.5, 31.5, 31.0, 27.4, 26.2, 26.1, 24.5, 24.4, 24.3, 24.2, 20.8, 19.9, 19.5, 16.1, 16.0, 15.6, 15.2, 14.2, 13.2, 11.7, 11.6, 10.0, 9.9 ppm.

2 5

Example 8.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=NHCH_2CH_2OH$; $R^{12}=-CH_2OH$.

The title compound was prepared from the title compound of Example 5 (0.6 g), 1-
3 0 hydroxybenzotriazole (0.09 g), aminoethanol (0.05 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.19 g) in N,N-dimethylformamide (5 mL) according to the procedure described in Example 6.
Yield: 0.52 g.

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Example 9.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=NHCH_2CH_2OH$; $R^{12}=-CH_2OH$.

5 The title compound was prepared from the title compound of Example 8 (0.49 g) and HF according to the procedures described in Example 7. Yield: 0.42 g; MS (FAB) m/z :

$M+K = 875$. m.p. 108-115°C.

^{13}C MR: ($CDCl_3$ -2 rotamers)

213.0, 212.9, 196.3, 193.1, 174.2, 169.5, 169.3, 165.6, 164.7, 138.9, 133.5,
133.2, 132.3, 130.9, 123.7, 123.4, 98.4, 97.7, 81.3, 80.4, 77.3, 77.0, 76.8,
10 76.4, 75.4, 73.6, 72.9, 72.4, 69.0, 62.3, 61.0, 60.9, 57.6, 57.2, 56.5, 56.3,
56.1, 55.3, 54.8, 53.0, 49.3, 48.6, 46.3, 44.1, 42.5, 42.3, 40.8, 40.0, 39.5,
38.8, 38.7, 35.0, 34.7, 34.1, 33.9, 33.8, 33.8, 33.6, 32.6, 32.5, 31.4, 31.1,
30.9, 27.2, 26.1, 26.1, 26.0, 24.5, 24.3, 24.2, 20.7, 19.9, 19.6, 16.0, 16.0,
15.7, 15.6, 14.0, 13.1, 11.7, 11.6, 10.1, 9.9 ppm.

15

Example 10.

Formula IIa: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Y=O$; $R^{11}=H$.

20 2-Fluoro-1-methylpyridinium p-toluenesulfonate (Mukaiyama reagent, 0.374 g) was added into a stirred solution of the title compound of Example 5 (0.6 g) in dichloromethane (35 mL) containing triethylamine (0.185 mL) at room temperature. After being stirred at room temperature overnight, solvent was removed *in vacuo*. The product was purified by silica gel chromatography (20g) eluting with 30% acetone in hexanes. Yield: 0.46 g; MS (FAB) m/z : $M+K = 928$.

25

Example 11.

Formula IIa: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Y=O$; $R^{11}=H$.

30 A solution of HF (0.045 mL of 48% aq HF in 2 mL of acetonitrile) was added into a stirred solution of the title compound of Example 10 (0.224 g) in acetonitrile (5 mL) at 0 °C. After being stirred at room temperature for 2 hours, the reaction was cooled to 0 °C and powdered sodium bicarbonate (1 g) was added and stirred for an additional hour. Solid was filtered off and solvent removed *in vacuo*. Product was purified by silica gel chromatography (4 g)

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eluting with 30% acetone in hexanes. Yield: 0.13 g; MS (FAB) m/z : $M+K = 814$. m.p. 115-118°C.

^{13}C NMR: (CDCl_3 -2 rotamers)

213.6, 213.5, 196.1, 192.4, 175.6, 169.0, 168.7, 165.8, 164.6, 139.7, 138.9,
 5 133.5, 133.0, 128.3, 128.3, 123.3, 123.0, 98.7, 97.1, 77.6, 77.2, 77.0, 76.9,
 76.7, 76.5, 75.2, 73.7, 73.6, 72.9, 72.2, 70.0, 68.9, 67.5, 57.7, 57.0, 56.6,
 56.3, 56.1, 55.0, 54.7, 52.8, 48.6, 48.5, 43.9, 43.3, 42.9, 40.2, 39.6, 39.3,
 38.8, 35.4, 35.4, 34.5, 33.7, 32.9, 32.9, 32.7, 32.5, 31.6, 28.9, 27.5, 26.4,
 26.2, 25.9, 24.6, 24.5, 24.4, 24.1, 22.6, 21.2, 20.8, 20.5, 19.5, 16.2, 16.0,
 10 15.9, 15.6, 14.3, 14.2, 14.1, 11.7, 9.8, 9.4 ppm.

Example 12.

Formula IIb: $n=1$; $R^1 = \text{Et}$; $R^2 = \text{H}$; $R^3 = \text{OTBS}$; $R^4 = \text{H}$; $R^5 = \text{H}$; R^6 and R^7 taken
 1 5 together = O; Z = n -Propyl-NH-; $R^{12} = -\text{CH}_2\text{OH}$.

The title compound was prepared from the title compound of Example 5 (0.6 g), 1-hydroxybenzotriazole (0.09 g), n -propylamine (0.05 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.19 g) in N,N -dimethylformamide (5 mL) according to the procedure described in Example 6.

2 0 Yield: 0.59 g.

Example 13.

Formula IIb: $n=1$; $R^1 = \text{Et}$; $R^2 = \text{H}$; $R^3 = \text{OH}$; $R^4 = \text{H}$; $R^5 = \text{H}$; R^6 and R^7 taken
 2 5 together = O; Z = n -Propyl-NH-; $R^{12} = -\text{CH}_2\text{OH}$.

The title compound was prepared from the title compound of Example 12 (0.39 g) and HF according to the procedures described in Example 7. Yield: 0.27 g; MS (FAB) m/z : $M+K = 873$. m.p. 98-102°C.

^{13}C NMR: (CDCl_3 -2 rotamers)

213.5, 212.7, 196.0, 192.4, 173.0, 173.0, 169.5, 169.3, 165.8, 164.5, 139.7,
 3 0 138.9, 133.6, 133.3, 131.6, 130.1, 123.5, 123.2, 98.7, 97.6, 80.4, 79.3,
 77.2, 77.0, 76.8, 76.4, 75.3, 73.6, 73.0, 72.4, 69.3, 68.5, 60.9, 60.9, 57.7,
 57.1, 56.5, 56.3, 56.0, 55.2, 54.9, 53.0, 49.1, 48.5, 45.4, 44.0, 43.6, 41.4,
 41.3, 40.1, 39.6, 39.4, 38.7, 38.6, 35.4, 34.6, 34.5, 33.8, 33.8, 32.5, 32.5,
 31.6, 31.4, 31.0, 27.3, 26.2, 26.1, 26.0, 24.6, 24.4, 24.3, 24.2, 22.8, 20.8,

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20.0, 19.4, 16.1, 16.0, 15.7, 15.6, 14.1, 13.4, 11.7, 11.6, 11.4, 10.0, 9.9 ppm.

Example 14.

5 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = morpholine; $R^{12}=-CH_2OH$.

The title compound was prepared from the title compound of Example 5 (0.6 g), 1-hydroxybenzotriazole (0.09 g), morpholine (0.1 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.19 g) in N,N-dimethylformamide (5 mL) according to
10 the procedure described in Example 6.

Yield: 0.53 g; MS (FAB) m/z : $M+K = 1015$

Example 15.

15 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = morpholine; $R^{12}=-CH_2OH$.

The title compound was prepared from the title compound of Example 12 (0.46 g) and HF according to the procedures described in Example 7. Yield: 0.32 g; MS (FAB) m/z : $M+K = 901$. m.p. 124-130°C.

13CNMR: (CDCl₃-2 rotamers) 213.6, 212.9, 196.1, 192.1, 171.7, 171.6,
20 169.3, 168.8, 165.8, 164.5, 139.6, 138.7, 133.6, 133.5, 130.9, 129.7, 123.2,
123.2, 98.6, 97.5, 79.7, 78.6, 77.4, 77.2, 77.0, 76.6, 76.4, 75.3, 73.6, 73.5,
72.9, 72.0, 69.5, 68.7, 66.8, 66.7, 66.7, 60.9, 60.9, 57.6, 57.0, 56.5, 56.3,
56.1, 55.1, 54.9, 52.9, 49.0, 48.6, 45.8, 44.7, 43.9, 43.4, 42.0, 40.0, 39.5,
39.4, 38.6, 35.5, 34.5, 34.2, 34.0, 33.5, 33.5, 32.6, 32.5, 31.6, 31.0, 30.5,
25 30.4, 30.2, 30.0, 27.3, 26.1, 25.8, 24.6, 24.5, 24.3, 20.9, 20.8, 20.1, 19.3,
16.1, 16.1, 15.7, 15.5, 14.6, 14.1, 13.7, 11.7, 11.6, 9.9, 9.9 ppm.

Example 16.

30 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = OH; $R^{12}=-CH_2NH-Bn$.

The title compound is prepared from the title compound of Example 4, benzylamine and sodium cyanoborohydride in acetic acid at 0 °C. The product is purified by reverse phase HPLC.

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Example 17.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH_2NH-Bn$.

- 5 The title compound is prepared from the title compound of Example 16 and hydrofluoric acid according to the procedure described in Example 7.

Example 18.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=morpholine$; $R^{12}=-CH_2NH-Bn$.

- 10 The title compound is prepared from the title compound of Example 17 and morpholine according to the procedure described in Example 6.

Example 19.

- 15 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH_2NH-Me$.

The title compound is prepared from the title compound of Example 3 and N-methylamine according to the procedure described in Example 16.

Example 20.

- 20 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=morpholine$; $R^{12}=-CH_2NH-Me$.

The title compound is prepared from the title compound of Example 19 and morpholine according to the procedure described in Example 6.

25 Example 21.

Formula IIc: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{13}=-CHO$.

- 30 Isobutyl chloroformate (3.3 mL) was added dropwise into a stirred solution of the title compound of Example 4 (19.2 g), triethylamine (3.5 mL) and water (18 mL) in acetone (200 mL) at 0 °C over 5 minutes. After being stirred at 0 °C for 1 hour, a solution of sodium azide (2.75 g) in water (50 mL) was added to the reaction at 0 °C and stirred at this temperature for additional 0.5 hour. The reaction mixture was partitioned between ether (650 mL) and aqueous sodium bicarbonate (sat'd, 3x150 mL). The organic phase was washed once brine (150 mL), dried over magnesium sulfate and solvent removed *in vacuo*

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(safety shield!). The crude acyl azide (19.6 g) was purified by silica gel chromatography (250 g) eluting with 20% acetone in hexanes. Yield: 17.4 g.

- Portion of the acyl azide (2 g) in dry tetrahydrofuran (30 mL) was refluxed under nitrogen for 3 hours. Solvent was removed *in vacuo* and product purified by silica gel chromatography. Yield: 2 g; MS (FAB) m/z : $M+K = 941$.

Example 22.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{13}=-CHO$.

- 1 0 The title compound was prepared from the title compound of Example 3 (0.23 g), isobutyl chloroformate, triethylamine and sodium azide according to the procedure described in Example 21. Yield: 0.17 g; MS (FAB) m/z : $M+K = 827$.

Example 23.

- 1 5 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH=CH_2$.

- Lithium bis(trimethylsilyl)amide (2.3 mL, 1.0 M in tetrahydrofuran) is added into a suspension of methyltriphenylphosphonium bromide (0.97 g) in dry toluene (7 mL) at 0 °C and stirred for 1 hour. A precooled solution of the product of Example 4 (0.7 g, in 14 mL of dry toluene) is added into the ylide over 7 minutes. After being stirred at 0 °C for 30 minutes, the reaction mixture is applied on a column of silica gel (50 g) in 40% acetone/hexanes and eluted with 40% acetone/hexanes to give the title compound.

Example 24.

- 2 5 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH=CH_2$.

The title compound is prepared from the title compound of Example 23 and hydrofluoric acid according to the procedure described in Example 7.

- 3 0 Example 25.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH_2CH_3$.

The title compound is prepared by hydrogenation of the title compound of Example 24 in the presence of Pd/C in ethanol.

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Example 26.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH(OH)CH_2(OH)$.

- 5 N-methylmorpholine N-oxide (0.73 g) and osmium tetroxide (1 mL, 4wt% in water) are added into a stirred solution of the product of Example 24 (0.7 g) in tetrahydrofuran (52 mL) and stirred at room temperature for 5 hours. Excess oxidizing agent is quenched with sodium bisulfite (0.9 g) and florisil. The reaction is then diluted with ethyl acetate and solid filtered off through celite. The filtrate is washed with 1 N sodium bicarbonate, brine,
 10 dried over magnesium sulfate and solvent removed *in vacuo*. The product is purified by silica gel chromatography.

Example 27.

- Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH=CHPh$.

The title compound is prepared from the title compound of Example 3 and benzyl-triphenylphosphonium bromide according to the procedure described in Example 23.

Example 28.

- 20 Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH_2CH_2Ph$.

The title compound is prepared from the title compound of Example 27 and hydrogen in the presence of Pd/C according to the procedure described in Example 25.

- 2.5 Example 29.

Formula IIc: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{13}=-CH=CH_2$.

The title compound is prepared from the title compound of Example 24, isobutyl chloroformate and sodium azide according to the procedure described in Example 21.

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Example 30.

Formula IIc: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{13}=-CH_2CH_3$.

- 5 The title compound is prepared from the title compound of Example 25, isobutyl chloroformate and sodium azide according to the procedure described in Example 21.

Example 31.

Formula IIb: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=OH$; $R^{12}=-CH_2OH$.

- 10 The title compound is prepared from the title compound of Example 5 and hydrofluoric acid according to the procedure described in Example 7.

Example 32.

Formula IIc: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{13}=-CH_2OH$.

- 15 The title compound is prepared from the title compound of Example 31, isobutyl chloroformate and sodium azide according to the procedure described in Example 21.

Example 33.

- 20 Formula IIId: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=morpholino$; $R^{13}=-CH_2CH_3$.

Morpholine (0.6 mL) is added into a stirred solution of the title compound of Example 30 (2.5 g) in dry tetrahydrofuran (25 mL) at room temperature for 1 hour. Solvent is removed *in vacuo* and product purified by silica gel chromatography.

25

Example 34.

Formula IIId: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=morpholino$; $R^{13}=-CH=CH_2$.

- 30 The title compound is prepared from the title compound of Example 29 and morpholine in dry tetrahydrofuran according to the procedure described in Example 33.

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Example 35.

Formula II d: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=BnNH-$; $R^{13}=-CH=CH_2$.

The title compound is prepared from the title compound of Example 29 and benzylamine in dry tetrahydrofuran according to the procedure described in Example 33.

Example 36a.

Formula II e: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O.

- 1 0 L-selectride (0.76 mL, 1 M solution in THF, Aldrich) was added dropwise into a stirred solution of the title compound of Example 21 (0.55 g) in dry tetrahydrofuran (5 mL) at -78 °C in ten minutes. After being stirred at -78 °C for 15 minutes, the reaction was partitioned between ether and 1 N hydrochloric acid. The organic phase was washed once with brine, dried over magnesium sulfate and solvent removed *in vacuo*. The product was
- 1 5 purified by silica gel chromatography (25 g) eluting with 30% acetone/hexanes. Yield: 0.26 g; MS (FAB) m/z : $M+K = 943$.

Example 36b.

Formula II d: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=H$; $R^{13}=-CH_2OH$.

- 2 0 The title compound was purified from the crude products of Example 36a. Yield: 0.04 g; MS (FAB) m/z : $M+K = 945$.

Example 36c.

- 2 5 Formula II d: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=H$; $R^{13}=-CH_2OH$.

The title compound is prepared from the title compound of Example 36b and hydrofluoric acid according to the procedure described in Example 7.

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Example 37.

Formula IIe: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O.

- 5 The title compound was prepared from the title compound of Example 36a and hydrofluoric acid according to the procedure described in Example 7. MS (FAB) m/z : $M+K = 829$.

Example 38.

- 10 Formula IIe: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $A=S$; $R^{14}=H$.

- The title compound of Example 4 (0.7 g) was dissolved in a solution of ethanol (freshly distilled from sodium, 5 mL) containing aminothioethanol hydrogen chloride (0.2 g) under nitrogen at room temperature. Diisopropylethylamine (0.14 mL) was added to the reaction mixture followed by powdered magnesium sulfate. After being stirred at room temperature for 1.5 hour, the solids were filtered off and solvent removed *in vacuo*. The intermediate was purified by silica gel chromatography. Yield: 0.56 g. The intermediate (0.56 g) was dissolved in dry THF (5 mL) under nitrogen at room temperature. Diisopropyl carbodiimide (0.18 mL), hydroxybenzotriazole (0.078 g) and N-methylmorpholine (0.096 mL) was added to the THF solution and stirred at room temperature overnight. Solvent was removed *in vacuo* and the product was purified by silica gel chromatography (13 g) eluting with 30% acetone in hexanes. Yield: 0.55 g.

Example 39.

- 25 Formula IIe: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $A=S$; $R^{14}=H$.

- The title compound was prepared by reacting the title compound of Example 38 (0.55 g) and hydrofluoric acid according to the procedure described in Example 7. Yield: 0.23 g; MS (FAB) m/z : $M+K = 871$. m.p. 117-125°C
 ^{13}C NMR: (CDCl₃-mixture of isomers each w/2 rotamers)
- 30 213.6, 213.5, 213.3, 213.2, 196.1, 196.0, 193.1, 192.4, 172.5, 172.4, 172.2, 169.0, 169.0, 168.9, 168.6, 165.8, 165.6, 164.7, 164.5, 139.7, 139.6, 138.9, 138.8, 134.6, 134.2, 133.1, 132.5, 128.6, 128.6, 126.4, 126.0, 123.4, 123.2, 123.1, 123.0, 98.7, 98.7, 97.2, 97.0, 78.2, 78.0, 77.5, 77.3, 77.0, 76.9, 76.8, 76.5, 76.3, 75.3, 75.2, 73.7, 73.6, 73.6, 72.9, 72.6, 72.1, 70.0, 69.8,

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69.1, 68.8, 61.2, 61.2, 58.2, 57.7, 57.5, 57.1, 57.0, 56.6, 56.6, 56.3, 56.1, 55.9, 55.0, 54.8, 54.7, 53.0, 52.7, 50.3, 50.2, 48.8, 48.6, 48.5, 48.5, 44.1, 43.8, 43.7, 43.7, 43.6, 43.3, 43.3, 42.8, 42.5, 42.4, 40.3, 40.1, 40.0, 40.0, 39.7, 39.6, 39.6, 39.4, 39.3, 36.6, 35.5, 35.1, 34.6, 34.5, 34.5, 34.0, 33.6, 33.2, 33.1, 32.9, 32.8, 32.7, 32.6, 32.6, 32.5, 31.6, 29.2, 29.2, 27.6, 27.6, 27.3, 26.4, 26.3, 26.2, 26.1, 26.0, 24.6, 24.6, 24.5, 24.4, 24.3, 24.2, 24.2, 22.6, 21.2, 21.1, 20.9, 20.8, 20.5, 20.4, 19.6, 19.4, 16.2, 16.0, 16.0, 15.9, 15.8, 15.7, 15.6, 14.4, 14.2, 14.1, 11.7, 10.1, 9.8, 9.4 ppm.

10

Example 40.

Formula II: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; A = O; $R^{14}=H$.

The title compound is prepared from the title compound of Example 3 and aminoethanol according to the procedure described in Example 38.

Example 41.

Formula II: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; A = S; R^{14} s taken together with the carbons they attached to = -Ph-

The title compound is prepared from the title compound of Example 3 and 2-aminothiophenol according to the procedure described in Example 38.

Example 42.

Formula II: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; A = NH; R^{14} s taken together with the carbons they attached to = -Ph-

The title compound is prepared from the title compound of Example 3 and o-phenylenediamine according to the procedure described in Example 38.

Example 43.

Formula II: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{15}=OH$.

Diphenylphosphoryl azide (0.8 mL) was added into a solution of the title compound of Example 4 (0.98 g) and triethylamine (0.26 mL) in dry tetrahydrofuran (3.5 mL) at room temperature. The reaction was stirred at 70 °C for 1 hour. After being cooled down to

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room temperature, the reaction mixture was poured to silica gel (25 g) and eluted with 10% acetone/hexanes followed by 30% acetone/hexanes. Yield: 0.34 g; MS (FAB) m/z : $M+K$ = 984.

5 Example 44.

Formula IIg: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{15}=OH$.

The title compound was prepared from the title compound of Example 3 and diphenylphosphoryl azide according to the procedure described in Example 43.

10 MS (FAB) m/z : $M+K$ = 870. m.p. 135-143°C.

^{13}C NMR:($CDCl_3$ -2 rotamers)

213.2, 196.1, 192.9, 169.0, 168.8, 165.9, 164.8, 154.3, 154.0, 139.6, 138.8,
134.1, 134.0, 133.6, 127.8, 124.3, 123.5, 123.2, 112.8, 112.7, 111.5, 111.4,
98.8, 97.2, 78.0, 78.0, 77.5, 77.2, 77.0, 76.8, 75.4, 73.9, 73.9, 73.1, 72.5,
15 70.0, 69.0, 65.8, 57.5, 57.0, 56.7, 56.2, 55.9, 55.1, 54.8, 52.9, 48.8, 48.6,
43.9, 43.7, 43.7, 43.4, 42.2, 42.1, 40.9, 40.6, 40.5, 40.0, 39.9, 39.3, 35.5,
34.6, 33.8, 33.2, 32.8, 32.7, 30.9, 28.2, 27.9, 27.5, 26.5, 26.4, 26.2, 24.6,
24.6, 24.4, 24.3, 21.2, 20.8, 20.5, 19.6, 16.2, 15.9, 15.8, 15.8, 15.2, 14.3,
14.2, 11.6, 9.9, 9.6 ppm.

20

Example 45.

Formula IIh: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O.

A solution of hydrofluoric acid (0.06 mL in 1 mL acetonitrile) was added into a stirred
25 solution of the title compound of Example 22 (0.16 g) in acetonitrile (1.5 mL) at 0 °C.
After being stirred at 0 °C for 2.5 hours, powdered sodium bicarbonate (0.5 g) was added
and stirred for another half hour. The reaction mixture was purified by C-18 reverse phase
HPLC eluting with aqueous acetonitrile containing 0.2% trifluoroacetic acid. Yield after
liophilization: 0.103 g; MS (FAB) m/z : $M+K$ = 783.

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Example 46.

Formula IIj: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O.

5 A suspension of 10% Pd/C in a solution of the title compound of Example 45 in tetrahydrofuran is stirred under an atmosphere of oxygen at room temperature for 10 days. Solids are filtered off and solvent removed *in vacuo*.

The product is purified by silica gel chromatography.

Example 47.

10 Formula IIj: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{16}=H$.

A solution of the title compound of Example 45 (32 mg) in methanol was hydrogenated under 1 atm of hydrogen in the presence of 5% Rh/Al₂O₃ for 2 hours. The product was purified by reverse phase HPLC. MS (FAB) *m/z*: M+K = 785.

Example 48.

15 Formula IIj: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{16}=H$.

20 The title compound is prepared from the title compound of Example 47 and hydrogen according to the procedure described in Example 47.

Example 49.

Formula IIj: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{16}=-CH_2Ph$.

25 Benzyl bromide is added into a stirred solution of the title compound of Example 47 and triethylamine in N,N-dimethylformamide. The product is purified by reverse phase HPLC.

Example 50.

30 Formula IIj: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{16}=methyl$.

The title compound is prepared from the title compound of Example 47, methyl iodide and triethylamine according to the procedure described in Example 49.

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Example 51.

Formula IIj: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{16}=-CH_2CH_2OH$.

- 5 The title compound is prepared from the title compound of Example 47, ethylene oxide and triethylamine according to the procedure described in Example 49.

Example 52.

Formula IIk: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=PhNH-$; $R^{15}=H$.

- 1 0 The title compound is prepared from the title compound of Example 47, phenyl isocyanate and triethylamine according to the procedure described in Example 49.

Example 53.

Formula IIk: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=morpholin-1-yl$; $R^{15}=OH$.

- 1 5 Morpholine (0.6 mL, neat) was added into a stirred solution of the title compound of Example 21 (2.5 g) in tetrahydrofuran (25 mL) at room temperature. After being stirred at room temperature for 0.5 hour, the solvent was removed *in vacuo* and the product purified by silica gel chromatography (150 g) eluting with 40% acetone/hexanes. Yield: 1.7 g; MS (FAB) m/z : $M+K = 1028$.
- 2 0

2 5 Example 54.

Formula IIm: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $Z=morpholin-1-yl$.

- Methanesulfonyl chloride (0.31 mL) was added into a stirred solution of the title compound of Example 53 (1.58 g) in dry tetrahydrofuran (16 mL) at 0 °C. Triethylamine (0.9 mL) was added dropwise to the reaction solution at 0 °C over 10 minutes. After being stirred for 1 hour, the reaction was partitioned between water and ether, the organic phase washed once with brine, dried over magnesium sulfate and solvent removed *in vacuo*. The product was purified by silica gel chromatography (50 g) eluting with 20% acetone/hexanes. Yield: 0.953 g; MS (FAB) m/z : $M+K = 1010$.
- 3 0

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Example 55.

Formula III: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OTBS$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = morpholin-1-yl; $R^{15}=H$.

- 5 A solution of the title compound of Example 54 (0.825 g) in methanol was hydrogenated over 1 atm of hydrogen in the presence of Rh/Al₂O₃ for 3 hours. The solids were filtered off and solvent removed *in vacuo*. The product was purified by silica gel chromatography (25 g) eluting with 25 % ethyl acetate/methylene chloride. Yield: 0.619 g.

10 Example 56.

Formula III: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = morpholin-1-yl; $R^{15}=H$.

- The title compound of Example 56 was prepared from the product of Example 55 (0.32 g) and hydrofluoric acid according to the procedure described in Example 7. The product
15 was purified by C-18 reverse HPLC eluting with aqueous acetonitrile containing 0.1% trifluoroacetic acid. Yield: 0.235 g; MS (FAB) *m/z*: M+K = 898.

Example 57.

Formula III: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = Ph; $R^{15}=H$.

- 20 The title compound is prepared from the title compound of Example 48 and benzoyl chloride in the presence of N-methylmorpholine.

Example 58.

- 25 Formula II: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O.

Aqueous phosphoric acid (1 wt %, 2 mL) was added into a stirred solution of the title compound of Example 21 (1.05 g) in dioxane (12 mL) and stirred at room temperature for 1.5 hour. The reaction mixture was partitioned between ether and sodium bicarbonate.

- 30 The organic phase was dried over magnesium sulfate and solvent removed *in vacuo*. The product was purified by silica gel chromatography (25 g) eluting with 18% acetone in hexanes. Yield: 0.35 g; MS (FAB) *m/z*: M+K = 941.

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Example 59.

Formula II m: $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; Z = morpholino.

The title compound is prepared from the title compound of Example 54 and hydrofluoric acid according to the procedure described in Example 7.

Example 60.

Formula II d': $n=1$; $R^1=Et$; $R^2=H$; $R^3=OH$; $R^4=H$; $R^5=H$; R^6 and R^7 taken together = O; $R^{12} = -CH=CH_2$.

The title compound is prepared from the title compound of Example 29 and hydrofluoric acid according to the procedure described in Example 7. The product is purified by reverse phase HPLC.

Example 61.1 5 In Vitro Assay of Biological Activity

The immunosuppressant activity of the compounds of the present invention was determined using the human mixed lymphocyte reaction (MLR) assay described by T. Kino *et al.* in *Transplantation Proceedings*, XIX(5):36-39, Suppl. 6 (1987). The results of the assay, shown below in Table 1, demonstrate that the compounds tested are effective immuno-modulators at low concentration.

Table 1

<u>Example #</u>	<u>IC₅₀ (M)</u>
7	23 x 10 ⁻⁹
9	65 x 10 ⁻⁹
11	1.1 x 10 ⁻⁹
13	388 x 10 ⁻⁹
15	25% at 1 x 10 ⁻⁶
37	0.57 x 10 ⁻⁹
39	37 x 10 ⁻⁹
44	75 x 10 ⁻⁹
56	2.6 x 10 ⁻⁹

2 5

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The compounds of the invention possess immunomodulatory activity in mammals (especially humans). The potent immunomodulatory activity which compounds of the instant invention demonstrate in common *in vitro* biological assays indicate that these compounds possess immunosuppressive, antimicrobial, antifungal, antiviral, 5 antiinflammatory, and antiproliferative activity, and possess the ability to reverse chemotherapeutic drug resistance. As agents which block T-cell activation, a prerequisite for HIV proliferation, the compounds are useful as prophylactics for the prevention of HIV replication. While the compounds of the invention are useful when used alone, combination therapy with other immunosuppressants, such as, FK506, rapamycin, 10 cyclosporin A, picibanil, mycophenolic acid, azathioprine, prednisolone, cyclophosphamide, brequinar and leflunomide, is also beneficial.

As immunosuppressants, the compounds of the present invention are useful when administered for the prevention immune-mediated tissue or organ graft rejection. Examples of transplanted tissues and organs which suffer from these effects are heart, 15 kidney, liver, medulla ossium, skin, cornea, lung, pancreas, intestinum tenue, limb, muscle, nervus, duodenum, small-bowel, pancreatic-islet-cell, and the like; as well as graft-versus-host diseases brought about by medulla ossium transplantation. The regulation of the immune response by the compounds of the invention means the compounds are useful in the treatment of autoimmune diseases, such as rheumatoid 20 arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I diabetes, uveitis, allergic encephalomyelitis, glomerulonephritis, and the like; and further infectious diseases caused by pathogenic microorganisms, such as HIV. In the particular cases of HIV-1, HIV-2 and related retroviral strains, inhibition of T-cell mitosis suppresses the replication of the virus, since the virus relies upon the host 25 T-cell's proliferative functions to replicate.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopic dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous 30 pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' ophthalmopathy, Vogt-

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5 Koyanagi-Harada syndrome, sarcoidosis, etc.; reversible obstructive airway disease, which includes conditions such as asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

10 Other treatable conditions include but are not limited to ischemic bowel diseases, inflammatory bowel diseases, necrotizing enterocolitis, intestinal lesions associated with thermal burns and leukotriene B₄-mediated diseases; intestinal inflammations/allergies such as Coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis; food-related allergic diseases which have symptomatic manifestation
15 remote from the gastro-intestinal tract (e.g., migraine, rhinitis and eczema); renal diseases such as interstitial nephritis, Goodpasture's syndrome, hemolytic-uremic syndrome and diabetic nephropathy; nervous diseases such as multiple myositis, Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy; endocrine diseases such as hyperthyroidism and Basedow's disease;
20 hematic diseases such as pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, agranulocytosis, pernicious anemia, megaloblastic anemia and anerythroplasia; bone diseases such as osteoporosis; respiratory diseases such as sarcoidosis, fibroid lung and idiopathic interstitial pneumonia; skin disease such as dermatomyositis, leukoderma vulgaris,
25 ichthyosis vulgaris, photoallergic sensitivity and cutaneous T cell lymphoma; circulatory diseases such as arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa and myocardosis; collagen diseases such as scleroderma, Wegener's granuloma and Sjogren's syndrome; adiposis; eosinophilic fasciitis; periodontal disease such as lesions of gingiva, periodontium, alveolar bone and substantia ossea dentis; nephrotic syndrome
30 such as glomerulonephritis; male pattern alopecia or alopecia senilis by preventing epilation or providing hair germination and/or promoting hair generation and hair growth; muscular dystrophy; Pyoderma and Sezary's syndrome; Addison's disease; active oxygen-mediated diseases, as for example organ injury such as ischemia-reperfusion injury of organs (such as heart, liver, kidney and digestive tract) which occurs upon preservation,

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transplantation or ischemic disease (for example, thrombosis and cardiac infraction): intestinal diseases such as endotoxin-shock, pseudomembranous colitis and colitis caused by drug or radiation; renal diseases such as ischemic acute renal insufficiency and chronic renal insufficiency; pulmonary diseases such as toxinoses caused by lung-oxygen or drug (for example, paracort and bleomycins), lung cancer and pulmonary emphysema; ocular diseases such as cataracta, siderosis, retinitis, pigmentosa, senile macular degeneration, vitreal scarring and corneal alkali burn; dermatitis such as erythema multiforme, linear IgA bullous dermatitis and cement dermatitis; and others such as gingivitis, periodontitis, sepsis, pancreatitis, diseases caused by environmental pollution (for example, air pollution), aging, carcinogenesis, metastasis of carcinoma and hypobaropathy; disease caused by histamine or leukotriene-C₄ release; Behcet's disease such as intestinal-, vasculo- or neuro-Behcet's disease, and also Behcet's which affects the oral cavity, skin, eye, vulva, articulation, epididymis, lung, kidney and so on.

Furthermore, the compounds of the invention are useful for the treatment and prevention of hepatic disease such as immunogenic diseases (for example, chronic autoimmune liver diseases such as the group consisting of autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g., necrosis caused by toxin, viral hepatitis, shock or anoxia), B-virus hepatitis, non-A/non-B hepatitis, cirrhosis (such as alcoholic cirrhosis) and hepatic failure such as fulminant hepatic failure, late-onset hepatic failure and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases), and moreover are useful for various diseases because of their useful activity such as augmentation of chemotherapeutic effect, preventing or treating activity of cytomegalovirus infection, particularly HCMV infection, anti-inflammatory activity, and so on.

Additionally, compounds of the invention possess rapamycin antagonistic properties, or wherein in the compounds of the invention R⁵ is -OH, FK-506 antagonistic properties. The compounds of the invention may thus be used in the treatment of immunodepression or a disorder involving immunodepression. Examples of disorders involving immunodepression include AIDS, cancer, senile dementia, trauma (including wound healing, surgery and shock), chronic bacterial infection, and certain central nervous system disorders. The immunodepression to be treated may be caused by an overdose of an immunosuppressive macrocyclic compound, for example derivatives of 12-(2-cyclohexyl-1-methylvinyl)-13, 19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}] octacos-18-ene such as FK-506, or rapamycin. Overdosing of such medicaments by

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patients is quite common upon their realizing that they have forgotten to take their medication at the prescribed time and can lead to serious side effects.

The ability of the compounds of the invention to treat proliferative diseases can be demonstrated according to the methods described in Bunchman ET and CA Brookshire, Transplantation Proceed. 23 967-968 (1991); Yamagishi, et al., Biochem. Biophys. Res. Comm. 191 840-846 (1993); and Shichiri, et al., J. Clin. Invest. 87 1867-1871 (1991). Proliferative diseases include smooth muscle proliferation, systemic sclerosis, cirrhosis of the liver, adult respiratory distress syndrome, idiopathic cardiomyopathy, lupus erythematosus, diabetic retinopathy or other retinopathies, psoriasis, scleroderma, prostatic hyperplasia, cardiac hyperplasia, restenosis following arterial injury or other pathologic stenosis of blood vessels.

Aqueous liquid compositions of the present invention are particularly useful for the treatment and prevention of various diseases of the eye such as autoimmune diseases (including, for example, conical cornea, keratitis, dysophia epithelialis corneae, leukoma, Mooren's ulcer, sclevitis and Graves' ophthalmopathy) and rejection of corneal transplantation.

When used in the above or other treatments, a therapeutically-effective amount of one of the compounds of the present invention, meaning a sufficient amount of the compound to treat a particular disorder, at a reasonable benefit/risk ratio, may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug form. Alternatively, the compound may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more pharmaceutically-acceptable excipients. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgement.

The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required

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to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

The total daily dose of the compounds of this invention administered to a human or lower animal may range from about 0.001 to about 10 mg/kg of patients body mass/day.

- 5 For purposes of oral administration, more preferable doses may be in the range of from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

- 10 The pharmaceutical compositions of the present invention comprise a compound of the invention and a pharmaceutically-acceptable carrier or excipient, meaning a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type, which may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. The term "parenteral" as used herein refers to
15 modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

- Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically-acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile
20 injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of
25 coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

- These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal
30 agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

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In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically-acceptable excipient or carrier, such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

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The solid dosage forms of tablets, dragees, capsules, pills, and granules may be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or
5 preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

10 Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl
15 benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as
20 wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions may contain, in addition to the active compounds, suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and
25 tragacanth, and mixtures thereof.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient in finely
30 divided form may be used in admixture with a larger-sized pharmaceutically-acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

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Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition is preferably such that the active ingredient does not dissolve therein to any substantial extent. The pressurized composition may also contain a surface active agent, such as a liquid or solid non-ionic surface active agent or may be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

A further form of topical administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as automimmune diseases, allergic or inflammatory conditions, and corneal transplants. The compound of the invention is delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically-acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material.

Compositions for rectal or vaginal administration are preferably suppositories which may be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Compounds of the present invention may also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically-acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*

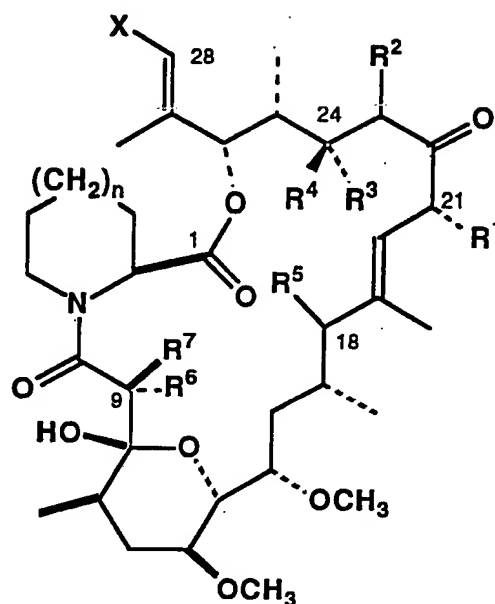
-50-

It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods of use of the invention, may be made without departing from the spirit and scope thereof.

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CLAIMSWhat is claimed is:

1. A compound of the formula:



(II)

- 5 wherein n is zero or one;

R¹ is selected from the group consisting of methyl, ethyl, propyl, cyclopropylmethyl, 2-oxopropyl, 2-ethanal, 2-hydroxyethyl and allyl;

- 10 R² is hydrogen or R² taken together with R³ forms a C-22/C-23 bond;

R³ is selected from the group consisting of hydrogen, hydroxy and protected hydroxy; or R² taken together with R³ forms a C-22/C-23 bond; or R³ taken together with R⁴ is oxo;

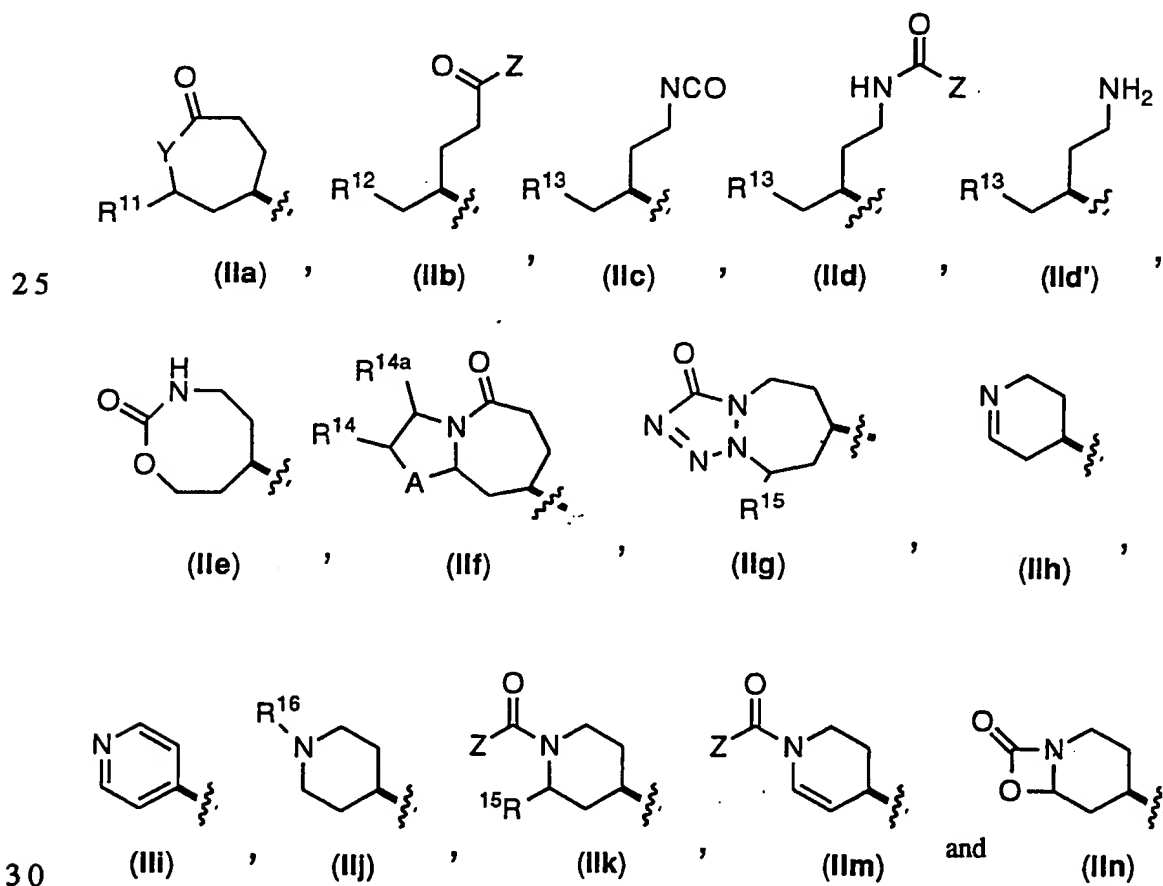
- 15 R⁴ is hydrogen or R⁴ taken together with R³ is oxo;

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R⁵ is selected from hydrogen, hydroxy, protected hydroxy and fluoro;

20 R⁶ and R⁷ are independently selected from hydrogen, hydroxy and protected hydroxy, with the proviso that at least one of R⁶ and R⁷ is hydrogen; or R⁶ and R⁷ taken together are oxo;

X is a substituent selected from the group of radicals having the subformulae:



wherein A is -O- or -S-;

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3 5 Y is -O- or -NR²¹- wherein R²¹ is selected from the group consisting of:

- (1) hydrogen,
- (2) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,
- (3) aryl and
- 4 0 (4) heterocyclic;

Z is selected from the group consisting of hydrogen, hydroxy, -(C1-C10-alkyl), aryl, heterocyclic, -(C1-C10-alkoxy) and -NR²³R²⁴, wherein R²³ and R²⁴ are independently selected from:

- 4 5 (1) hydrogen,
- (2) aryl,
- (3) heterocyclic and
- (4) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,

5 0

or, alternatively, -NR²³R²⁴ represents a 3- to 7-membered heterocyclic ring, with the proviso that in subformulae (II_d), (II_k) and (II_m) Z is other than hydroxy;

R¹¹ is hydrogen, hydroxy or methoxy;

5 5

R¹² is selected from the group consisting of:

- (1) -CHO,
- (2) -(C1-C10-alkyl) optionally substituted with one, two or three substituents independently selected from the group consisting of hydroxy, amino, aryl and heterocyclic,
- 6 0 (3) -(C2-C10-alkenyl) optionally substituted with aryl or heterocyclic,
- (4) -CH₂-NR²¹R²² where R²¹ is independently defined as above and R²² is hydrogen or acyl;

6 5 R¹³ is selected from the group consisting of -CHO, hydroxy, protected hydroxy, -(C2-C10-alkenyl) optionally substituted with aryl and -(C1-C10-alkyl) optionally substituted with aryl, with the proviso that in subformula (II_d) R¹³ is other than -CHO;

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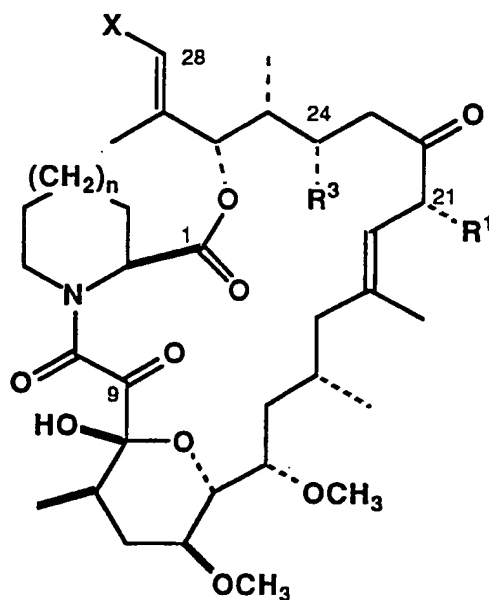
7 0 R^{14} and R^{14a} are independently selected from hydrogen, aryl, heterocyclic and -(C1-C10-alkyl) or, taken together with the carbon atoms to which they are attached, R^{14} and R^{14a} form an aryl group or a heterocyclic group;

R^{15} is hydrogen or hydroxy; and

7 5 R^{16} is selected from the group consisting of
 (1) hydrogen,
 (2) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or heterocyclic,
 (3) aryl,
 8 0 (4) heterocyclic and
 (5) -C(=NH)-NH₂;

or a pharmaceutically acceptable salt, ester, amide or prodrug thereof.

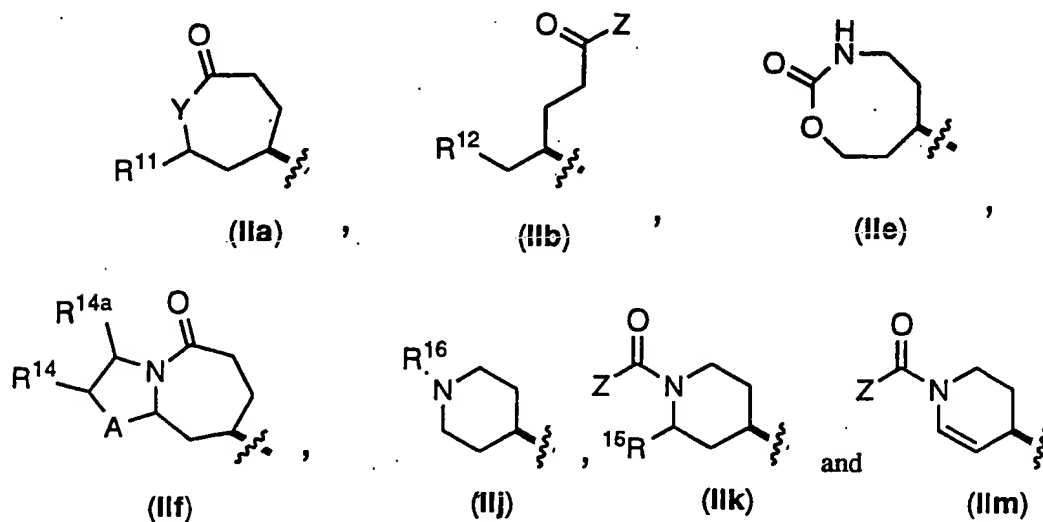
2. A compound according to Claim 1 of the formula



(III)

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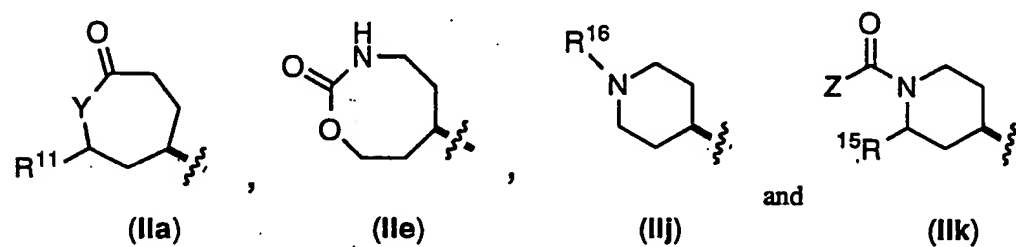
- 5 wherein n is one, R¹ and R³ are as defined therein and X is a substituent selected from the group of radicals having the subformulae:



10

wherein A, Y, Z, R¹², R¹⁴, R^{14a} and R¹⁶ are as defined therein and R¹¹ and R¹⁵ are each hydrogen.

3. A compound according to Claim 2 wherein X is a substituent selected from the group of radicals having the subformulae:



5

wherein Y, Z and R¹⁶ are as defined therein and R¹¹ and R¹⁵ are each hydrogen.

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4. A compound according to Claim 3 wherein wherein Y is -O-,
Z is -(C1-C10-alkyl), aryl, heterocyclic or -NR²³R²⁴, wherein R²³ and R²⁴ are
independently selected from:

- 5 (1) hydrogen,
 (2) aryl,
 (3) heterocyclic and
 (4) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or
 heterocyclic,

or, alternatively, -NR²³R²⁴ represents a 3- to 7-membered heterocyclic ring.

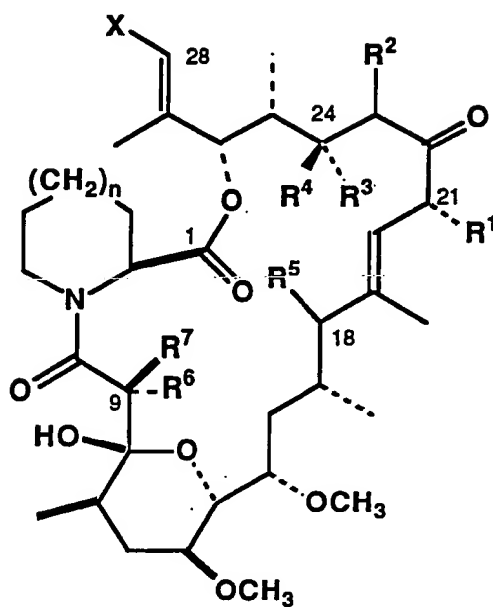
10 R¹⁶ is

- (1) hydrogen,
 (2) -(C1-C10-alkyl) optionally substituted with hydroxy, amino, aryl or
 heterocyclic,
 (3) aryl or
15 (4) heterocyclic,
and R¹¹ and R¹⁵ are each hydrogen.

5. A compound according to Claim 4 wherein Y is -O- and Z is -CH₃ and R¹¹ and
R¹⁵ are each hydrogen.

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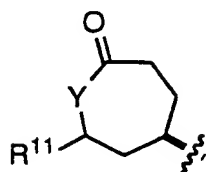
6. A compound of the formula:



(II)

5

(a) wherein $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OH$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; and X is



(IIa)

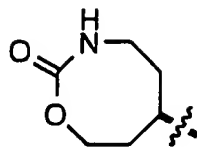
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wherein Y = O; and $R^{11} = H$;

(b) wherein $n=1$; $R^1 = Et$; $R^2 = H$; $R^3 = OH$; $R^4 = H$; $R^5 = H$; R^6 and R^7 taken together = O; and X is

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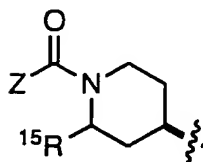
15



(Ile) ; or

(c) wherein $n=1$; $R^1 = \text{Et}$; $R^2 = \text{H}$; $R^3 = \text{OH}$; $R^4 = \text{H}$; $R^5 = \text{H}$; R^6 and R^7 taken together = O; and X is

20



(IIk)

wherein Z = morpholin-1-yl; $R^{15} = \text{H}$;
or a pharmaceutically acceptable salt, ester, amide or prodrug thereof.

7. A pharmaceutical composition for immunomodulatory treatment comprising a compound according to Claim 1 in combination with a pharmaceutically acceptable carrier.

8. A pharmaceutical composition for immunomodulatory treatment comprising a compound according to Claim 3 in combination with a pharmaceutically acceptable carrier.

9. A pharmaceutical composition for immunomodulatory treatment comprising a compound according to Claim 6 in combination with a pharmaceutically acceptable carrier.

10. A method of immunomodulatory treatment comprising administering an effective amount of a compound according to Claim 1 to a patient in need of such treatment.

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11. A method of immunomodulatory treatment comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to Claim 1.

12. A method of immunomodulatory treatment comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to Claim 3.

13. A method of immunomodulatory treatment comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to Claim 6.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/13210

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D498/18 A61K31/535 A61K31/445 C07H19/01
/(C07D498/18,311:00,273:00,221:00),(C07D498/18,313:00,311:00,
273:00,221:00),(C07D498/18,311:00,273:00,267:00,221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 04679 (ABBOTT LAB) 18 March 1993 * see in particular the definition of the substituent X in claim 1 and examples 408 - 490 *	1-13
Y	WO,A,93 04680 (ABBOTT LAB) 18 March 1993 * see in particular the definition of X * see claim 1	1-13
Y	GB,A,2 244 991 (FUJISAWA PHARMACEUTICAL CO) 18 December 1991 see claim 1	1-13
Y	WO,A,91 13889 (FISONS PLC) 19 September 1991 see claim 1	1-13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 March 1995

Date of mailing of the international search report

11.05.95

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Stellmach, J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/13210

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	EP,A,0 532 089 (MERCK & CO INC) 17 March 1993 see claim 1 ---	1-13
Y	EP,A,0 480 623 (MERCK & CO INC) 15 April 1992 see claim 1 ---	1-13
Y	EP,A,0 428 365 (MERCK & CO INC) 22 May 1991 * see the definition of R1 and R2 * see claim 1 ---	1-13
Y	EP,A,0 427 680 (SANDOZ LTD ;SANDOZ AG (DE); SANDOZ AG (AT)) 15 May 1991 see claim 1 ---	1-13
Y	IMMUNOLOGY TODAY, vol. 13, no.4, 1992 pages 136-142, SCHREIBER,S.L., CRABTREE,G.R. 'The mechanism of action of Cyclosporin A and FK 506' see the whole document ---	1-13
Y	J.MED.CHEM., vol. 35, no.23, 1992 WASHINGTON, pages 4284-4296, HAUSKE,J.R. ET AL. 'Design and Synthesis of Novel FKBP inhibitors' * see in particular page 1290, fig. 2 * ---	1-13
Y	SCIENCE, vol. 251, 18 January 1991 WASHINGTON, pages 283-287, SCHREIBER,S.L. 'Chemistry and Biology of the Immunophilins and Their Immunosuppressive Ligands' see the whole document ---	1-13
P,Y	TETRAHEDRON, vol. 50, no.3, 1994 OXFORD, pages 809-826, BATCHELOR,M.J. ET AL. 'Total Synthesis of Close analogues of the Immunosuppressant FK 506' * see page 819, scheme 1 * ---	1-13

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	<p>TETRAHEDRON, vol. 50, no.48, 1994 OXFORD, pages 13655-13670, ZIMMER,R. ET AL. 'Synthetic Modifications of Asomycin- I. A Chemoselective Removal of the Cyclohexyl Residue of Asomycin' see the whole document -----</p>	1-13

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Information on patent family members

International Application No
PCT/US 94/13210

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		AU-A- 2667392	05-04-93
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		JP-T- 6509583	27-10-94
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		AU-A- 2667392	05-04-93
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		JP-T- 6509583	27-10-94
		WO-A- 9304679	18-03-93
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International Application No

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